The reproductive ecology of two corals and one gorgonian from sub-tropical Bermuda.

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The Reproductive Ecology of two corals and one gorgonian from sub-tropical Bermuda

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Summary

This study examines the sexual reproductive ecology of three species of corals common on the sub-tropical reefs of Bermuda: the scleractinians *Porites astreoides* and *Madracis mirabilis*, and the gorgonian *Pseudoplexaura porosa*. The reproductive biology of corals in Bermuda is of particular interest because low winter seawater temperatures and geographical isolation make it an extreme of distribution for many of the species there. In addition, the Bermuda platform comprises reef zones that experience gradients of temperature, sediment loading and wave intensity at the different depths. The objectives are twofold: firstly, information is presented on the reproductive mode, sexuality and fecundity of the corals, and the occurrence of lunar periodicity to gamete development, planula release or spawning. The second objective addresses the question of whether environmental variability across the Bermuda platform and between years alters the reproductive cycles of these species.

The study species varied in reproductive mode and sexuality. The scleractinian *Porites astreoides* is a brooder with a mixed sexuality; the gorgonian *Pseudoplexaura porosa* exhibits gonochorism with broadcasting, and the scleractinian *Madracis mirabilis* has hermaphroditic colonies with a proposed intermediate 'pseudo-brooding' reproductive mode. Fecundity was variable within and between *Po. astreoides* colonies but was not related to colony size. There was a relationship between polyp size and gamete production in *Ps. porosa*. The synchrony of lunar periodicity to spawning or planulae release varied between the species and this is related to the different reproductive modes. The extent that planula release of *Po. astreoides* was synchronised to the lunar cycle also varied according to the reef zone in Bermuda, a proposed consequence of variable turbidity levels from inshore to offshore. Observed differences in the reproductive effort of *Po. astreoides* and *Ps. porosa*, both at the different reef zones within Bermuda, as well across study years, are related to spatial and inter-annual variations in temperature profiles.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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Chapter 1: Introduction

1.1 Overview

An understanding of coral reproductive cycles is an important component of our knowledge base on the ecological processes of coral reefs and, as such, is necessary for their management and preservation. Successful coral reproduction is essential for the addition of new genets in an area, the colonisation of new areas and the regeneration of areas destroyed by natural or human disturbances. In the last few decades, threats to coral reef areas have increased both directly, through increased human population density and industrialisation, and indirectly via associated climate changes (reviews by Richmond, 1993; Brown, 1997; Hodgson, 1999; Hughes and Connell, 1999; Souter and Linden, 2000). Unfortunately, sublethal stress caused by changes in ambient environmental conditions has been shown to reduce or cease reproductive activities in corals as resources are diverted to other life functions (Kojis and Quinn, 1983; Tomascik and Sander, 1987; Harrison and Wallace, 1990; Richmond, 1997; Ward and Harrison, 2000; Harrison and Ward, 2001; Cox and Ward, 2002). An understanding of coral reproductive cycles and the influence of environmental factors on reproduction is therefore essential in comprehending the potential susceptibility of this sensitive and critical part of the life cycle.

Seawater temperature is believed to be the crucial environmental factor limiting hermatypic coral reefs to the tropical and sub-tropical environment (Yonge, 1940; Wells, 1957). An increased knowledge of reproduction in coral reef systems at their temperature defined distribution extremes will therefore provide information as to the role of temperature on coral reproduction. Furthermore, recent changes in seawater temperature have been reported on a global scale, and it is clear that thermal cycles are not stable (Houghton *et al.*, 1996). An understanding of the control exerted by temperature on coral reproduction is thus needed to ascertain any effects of global climate changes on coral populations.

This study examines the sexual reproductive ecology of three species of corals common on the high latitude reefs of Bermuda: the scleractinian species *Porites astreoides* and *Madracis mirabilis*, and the gorgonian *Pseudoplexaura porosa*. No previous studies on the reproduction of these species in Bermuda have been published. Indeed, published studies on coral reproduction in Bermuda are limited to only four scleractinian species (Wyers, 1985; Wyers *et al.*, 1991), although the sub-tropical reefs support 21 scleractinian and 19 gorgonian species (Sterrer, 1986). At latitude 32°N, Bermuda is the most northerly coral reef in the Atlantic, and the low winter seawater temperatures and its geographical isolation make Bermuda an extreme of distribution for many species. The geology, structure and environment of the Bermuda islands and a description of the coral reefs are presented in Chapter 2. Environmental parameters vary across the Bermuda platform, which can be divided into at least three physiographic reef zones: the Inner Lagoon, Outer Lagoon and Rim Reef, which exhibit gradients of temperature, turbidity and wave intensity (Chapter 2). The reproductive ecology of the three study species is examined across these different reef zones to provide information on the effects of this inter zone environmental variation on the coral populations.

The reproductive cycles of *Porites astreoides* and *Pseudoplexaura porosa* in Bermuda are documented in Chapter 3 and 4 respectively. Information is presented on the sexuality of the corals and the population, variations in fecundity within and between colonies, patterns of gametogenesis, and lunar periodicity to gamete development, planula release or spawning. Additional information is provided on the population ecology of *Ps. porosa* as information on gorgonian ecology in Bermuda is limited.

In Chapter 5, the timing and duration of the reproductive cycle of *Po. astreoides* and *Ps. porosa* is related to the annual temperature range in Bermuda. Comparisons are made between the reproductive season of these species in Bermuda with available information from the Caribbean. Observed differences in the reproductive effort of the species, both at the different reef zones within Bermuda, as well across the study years, are related to spatial and inter-annual variations in temperature profiles.

Chapter 6 documents the reproductive cycle of *Madracis mirabilis* in Bermuda, presenting information on the gametogenic cycle, fecundity and the relationship between lunar periodicity and gamete development. Comparisons and contrasts are made with a recent study on the reproductive cycle of this species in the Southern Caribbean (Vermeij *et al.*, 2003; Vermeij *et al.*, in review). The reproductive mode of *M. mirabilis* is still under discussion, although evidence is presented for a "pseudo-brooding" or a mixed reproductive strategy of both brooding and broadcasting.

The following introductory chapter is primarily an account of the reproductive ecology of scleractinian and gorgonian coral species. Summary information on other anthozoan orders is included where relevant.

1.2 Coral reproductive strategy

Corals can undergo both sexual and asexual reproduction. An important mode of asexual proliferation and formation of new colonies in several coral species is fragmentation (see review by Highsmith, 1982, for scleractinian corals, and Lasker, 1983; Walker and Bull, 1983; Lasker, 1984, for gorgonian species). Fragmentation occurs at a number of levels, ranging from rare and stochastic to a primary feature incorporated into the life history. For example, it was estimated that 94% of colonies of a population of the gorgonian *Plexaura kuna* had arisen from fragmentation (Lasker, 1984, as *Plexaura* A). The occurrence of fragmentation in a coral species is determined by both morphology and habitat, as it is usually initiated from a structural weakness in the colony and then completed by physical forces, such as storm action. Some gorgonian species show adaptations to enhance fragmentation, for example autonomy of branch ends of the sea whip, *Junceella fragilis* (Walker and Bull, 1983), and the axis constrictions along branches of *Plexaura kuna* that enhance breaking (Lasker, 1984). Fragmentation in scleractinian corals primarily occurs in species with branching growth form as this morphology is susceptible to breakage and the fragments are easily propagated (Bothwell, 1981; Highsmith, 1982). On the high energy reef flat environments of many Pacific reefs, branching species with prolific fragmentation, such

as some *Acropora* (Connell, 1973; Stimson, 1978) and *Pocillopora* (Richmond, 1985) species dominate the area.

The larger sized fragment recruits have a lower rate of mortality and higher competitive ability compared to sexually produced juvenile colonies and so have the potential to dominate an area (Hughes and Jackson, 1980; Babcock, 1988; Lasker, 1990). Thus, fragmentation leads to a high rate of reef growth and reef-frame production (Highsmith, 1982). Recruitment by fragmentation is also important for recovery from disturbances as re-establishment occurs over a shorter time scale compared to the slow recruitment of sexual propagules. Dispersal by fragmentation, however, is limited and will lead to a patchy distribution of colonies (Wallace, 1985). A further repercussion of asexual reproduction is the proliferation of a single genome, potentially of one sex (Kojis and Quinn, 1981a). The resultant low levels of genetic variation through asexual reproduction will bestow the same level of success on the offspring of that of the parent, which will allow for proliferation under stable conditions but may limit population adaptability and survival under periods of change (Richmond, 1997).

Another form of asexual reproduction is the formation of 'polyp balls', reported from *Goniopora stokesi* (Rosen and Taylor, 1969). A spherical area of polyps with skeleton develops on the surface of adult colonies and then becomes detached, drops off and establishes on the surrounding reef. Asexual reproduction also occurs by 'polyp bail-out', a process whereby single polyps, without any skeleton, are expelled from colonies, drift in the water current and become attached elsewhere (Sammarco, 1982; Richmond, 1985). The latter is reported to have occurred as a stress response in some pocilloporid coral species. A similar mechanism is 'coral polyp expulsion', in which the polyp is expelled with its calyx. This process apparently occurs in healthy corals, and has been reported from *Favia favus* in the Red Sea and *Oculina patagonica* in the Mediterranean (Kramarsky-Winter *et al.*, 1997). Coral larvae, which are the normal product of sexual reproduction, can also be produced asexually, as has been recorded for *Pocillopora damicornis* (Stoddart, 1983; Ward, 1992), *Tubastrea coccinea* and *T. diaphana* (Ayre and Resing, 1986).

Sexual reproduction in corals comprises the process of gametogenesis followed by the successful fusion of eggs and sperm and the formation of a planula which, after a dispersal

period, may find a suitable habitat to settle resulting in recruitment to the population. Corals are similar to many lower marine invertebrates in that they lack gonad structures. The gametes develop directly within the mesenteries or on stalks attached to the mesenteries, enveloped by mesoglea and endodermis. Primordial germ cells develop from the endodermis and then migrate into the mesoglea. The round oocytes occur singly or in groups or strings along the mesenteries, and sperm develop within spermaries which are often also spherical or oval in shape. Mature gametes are fertilised either internally or externally, leading to differences in the location of the subsequent embryonic development of the planula. The location of embryonic development is used as a descriptor of the reproductive mode of a species, with species being described as broadcasting or brooding. A broadcasting mode involves the release of gametes from a coral colony by spawning followed by external fertilisation and with planula development occurring entirely in the water. Brooding involves internal fertilisation, and the development of the planulae takes place inside the 'parental' polyp before release into the water, a process known as planulation. A variation is surface brooding of embryos on the colony surface after fertilisation either internally or at the colony surface, as reported in the gorgonian corals *Paramuricea clavata* (Coma *et al.*, 1995a) and *Briareum asbestinum* (Brazeau and Lasker, 1990), two alcyonacean and two stoloniferan species (see review in Benayahu *et al.*, 1990), and an actinarian species (Dunn, 1975). A further modification to the originally defined coral reproductive modes is the proposed "quick-releasing" strategy of internal fertilisation followed by a short or no brooding period (de Graaf *et al.*, 1999; Vermeij *et al.*, in review), and this is discussed in Chapter 6.

The internal brooding of planulae was historically thought to be the dominant mode of reproduction in scleractinian corals (see reviews by Marshall and Stephenson, 1933; Fadlallah, 1983a; Szmant, 1986; Harrison and Wallace, 1990; Richmond, 1997). The techniques used in early studies detected planulation more readily than gamete release and, when the majority of species studied were shown not to produce planulae, this led to the false conclusion that they were sterile and that viviparity was the rule in reproductive colonies (Harrison and Wallace, 1990). Subsequent research on scleractinian coral reproduction has confirmed the wide occurrence of the broadcasting mode of reproduction. By 1990, a review of Pacific corals covering the Great Barrier Reef, Central Pacific, Hawaii and Japan, documented 198 species as broadcasters while

only 28 of the species studied brooded planulae (review by Richmond and Hunter, 1990). Broadcasting is also the dominant mode of reproduction reported in the Red Sea, with only three out of the 24 species for which reproductive method is known, brooding planulae (Shlesinger *et al.*, 1998). The Caribbean is the exception to this pattern, with a brooding mode of reproduction now confirmed for 13 species of scleractinians and a similar number of species known to broadcast spawn (Table 7.1, Chapter 7). Globally, approximately 250 coral species are now known to spawn gametes while 66 species are known to brood planulae (P. Harrison, pers. comm.).

In comparison to the plethora of research on scleractinian corals, information on gorgonian reproductive cycles and modes is scarce. Early studies in the Caribbean are limited to the documentation of a few Caribbean species (Wilson, 1883 cited from Goldberg and Hamilton, 1974; Cary, 1914, cited from Goldberg and Hamilton, 1974). Kinzie (1970) studied the ecology of gorgonian populations in Jamaica, including the life history and larval ecology of *Pseudopterogorgia bipinnata*. In the early 1970s there was an interest in the Caribbean plexaurid *Plexaura homomalla*, as it was found to contain significant amounts of a pharmaceutical prostaglandin (Weinheimer, 1974). The research focused on the practicalities of commercial harvesting of this species including many biological and ecological aspects (Bayer, 1974; Kinzie, 1974) but studies on the sexual cycle were unfortunately limited (Goldberg and Hamilton, 1974). The increase in gorgonian research in the Caribbean over the last 20 years is largely attributable to H.R. Lasker, D.A. Brazeau and colleagues working from the Smithsonian Tropical Research Institute field station in the San Blas Islands of Panama. Their research has documented the population dynamics, ecology, reproductive cycle and fertilisation success of the abundant plexaurid gorgonian *Plexaura kuna* (Lasker, 1984; Brazeau and Lasker, 1989; Lasker, 1990; Lasker, 1991; Lasker and Stewart, 1992; Lasker *et al.*, 1996a; Lasker *et al.*, 1996b, Lasker and Kim, 1996; Lasker *et al.*, 1998) and the reproductive cycle and fertilisation success of *Briareum asbestinum* (Brazeau and Lasker, 1990; Brazeau and Lasker, 1992). Coma and Lasker (1997a) researched the fertilisation rates of *Pseudoplexaura porosa*, also documenting the reproductive cycle. Kapela and Lasker (1999) further studied the gametogenic cycle of *P. porosa* and the relationship between colony size and reproductive effort. A recent paper recorded the reproductive cycle and egg production in *Plexaura flexuosa* (Beiring and Lasker, 2000). H. Lasker also has research in progress on the reproductive cycle of *Pseudopterogorgia*

species and *Plexaurella* sp. Research on gorgonian reproduction outside of the Caribbean is limited to a few species (Grigg, 1977; Grigg, 1979; Coma *et al.*, 1995a; Coma *et al.*, 1995b).

The mode of reproduction is known only for a limited number of gorgonians. Broadcast spawning of gametes has been confirmed from eight species, internal brooding occurs in three species, and is suspected from a further two species, and external brooding of embryos on the colony surface occurs in four species. The closely related alcyonaceans (also subclass Octocorallia) similarly exhibit a variety of reproductive modes. Of the studied species by 1990, 23 were broadcast spawners, 18 were brooders and two species exhibited surface brooding (Alino and Coll, 1989; Benayahu *et al.*, 1990). Little reproductive research has been carried out on the other common orders of the class Octocorallia. External brooding is known from the Stolonifera (cited from Benyahu *et al.*, 1990), and broadcast spawning from the Pennatulacea (Eckelbarger *et al.*, 1998). Other common orders of the subclass Hexacorallia for which reproductive studies have been made are the Zoanthidea and Actiniaria. A broadcasting reproductive mode dominates in the zoanthids, with only one species known to internally brood planulae (Ryland, 1997). The majority (~82%) of the anemone species studied are also broadcast spawners, with at least three species known to brood larvae and three species have direct development (Chia, 1976; Table 6.1 in Shick, 1991).

The reproductive mode of coral species, and the associated differences in the period of larval development, determines dispersal capability. The planktonic development of the planulae of broadcasting species to settlement generally takes 4-6 days or longer (Shlesinger and Loya, 1985; Babcock and Heyward, 1986; Lasker and Kim, 1996; Sakai, 1997). In contrast, brooded planulae may be competent to settle immediately on release (Fadlallah and Pearse, 1982a), within a few hours (Lewis, 1974b; Goreau *et al.*, 1981) to in one or two days (Atoda, 1951; Rinkevich and Loya, 1979a; Kojis and Quinn, 1982; Tranter *et al.*, 1982; Van Moorsel, 1983; Shlesinger and Loya, 1985; Szmant-Froelich *et al.*, 1985). However, some brooded planulae retain their competency for extended periods. The planulae of *Pocillopora damicornis* are able to spend 100 days in the plankton, accounting for the wide distribution of this species across the entire Pacific, Red Sea and Indian Ocean (Richmond, 1988). The more

typical short dispersal time associated with brooding has been suggested as a mechanism for retaining propagules if the parental habitat is either favourable (Szmant, 1986) or patchy (Kojis and Quinn, 1982; Harriott, 1992). In such areas, dispersal would move the propagules into inhabitable areas. Further relating reproductive mode and dispersal to habitat, Stimson (1978) suggested that corals preferentially inhabiting shallow-water reef flats where there is characteristically fast moving water would also adopt a brooding mechanism with low dispersal in order to promote the retention of their planulae in the immediate area. Broadcast spawning species also occur on reef flats and some of them encourage the retention of their planulae by releasing negatively buoyant eggs during low tide when the current is least (for example, *Fungia scutaria*, Krupp, 1983; and *Goniastrea favulus*, Kojis and Quinn, 1981b; Kojis and Quinn, 1982). However, other studies have found no evidence that relates reproductive mode and dispersal capability to habitat conditions (Kojis and Quinn, 1981a; Fadlallah and Pearse, 1982a; Van Moorsel, 1983; Harriott, 1983a; Babcock, 1984; Glynn *et al.*, 1991).

Reproductive mode and dispersal time also affect the survival and fitness of the planulae produced by a given coral species. The long planktonic periods associated with external development often result in higher mortality than the short dispersal period of brooded planulae, as planulae are susceptible to predation and environmental change in the water column and may also be swept away from favourable areas. The planulae of brooding corals also have an energetic advantage over planulae from broadcasting species as the former usually contain zooxanthellae when they are released (Harrison and Wallace, 1990), although there are some known exceptions (Krupp, 1983; Rinkevich, 1989). There are limited reports of the oocytes of scleractinian broadcasting species incorporating zooxanthellae (Kojis and Quinn, 1981a; Glynn *et al.*, 1991; Kruger and Schleyer, 1998), and externally developing larvae generally incorporate algal symbionts during the planktonic phase or when settlement and metamorphosis has occurred. The studied gorgonian species with a broadcasting reproductive mode do not incorporate zooxanthellae into oocytes, and neither do the majority of alcyonaceas (Fabricius and Alderslade, 2001), with a few known exceptions (for example, Benayahu *et al*,. 1992). Similarly, there is only one example of vertical transmission occurring in a zoanthid (Ryland, 1997). Thus, in general, recruitment efficiency can be enhanced by a brooding reproductive mode (Szmant-Froelich *et al.*, 1985; Richmond, 1997), which has been reflected in the greater number of recruits recorded from brooding species in

the Caribbean in comparison to broadcasting species (Bak and Engel, 1979; Rylaarsdam, 1983; Smith, 1992). However, post-settlement mortality is often greater in brooding species, and many communities in the Caribbean are dominated by broadcasting species which are long-lived, massive corals (Smith, 1992).

Contributing to the increased recruitment of species with a brooding mode is the fact that, for many, the breeding season commonly extends over several months, compared to the brief spawning periods of broadcasting species. It has therefore been suggested that brooding would be selected for in species inhabiting dynamic, unstable environments with high levels of disturbance, as a mechanism providing continuous replenishment in such areas of high adult mortality (Stimson, 1978; Szmant, 1986). The predictability of the disturbance will determine the length of the breeding season, with species inhabiting areas of frequent, unpredictable disturbance producing many small propagules over a long period (Van Moorsel, 1983). The prolonged release of propagules decreases the chances of the reproductive effort being destroyed by a stochastic environmental event. An example is provided by the Caribbean genus *Agaricia*: one species, *A. humilis*, predominantly lives in shallow, dynamic waters and releases planulae year round, whereas *A. agaricites* inhabits a deeper, more stable environment and planulate over just a few months (Van Moorsel, 1983).

The brooding of planulae is often correlated with an 'r-selected' life-history strategy of 'live fast and die young', with a large allocation of energy devoted to reproduction at an early age (Loya, 1976). Consequently, many brooding species have a smaller colony size than broadcasting species, as energy is channelled into reproduction rather than growth (Goreau *et al.*, 1981; Kojis and Quinn, 1981a; Kojis and Quinn, 1982; Van Moorsel, 1983; Szmant, 1986; Soong, 1993). In comparison, many broadcasting species have the opposite attributes and exhibit a 'K-selected' lifestyle with a large colony and delayed age at first reproduction. Coral polyp size is likewise correlated with reproductive mode in some species. The size constraints of small colonies and/or small polyps cannot accommodate enough gametes to offset the high wastage associated with broadcasting. These species benefit more from allocating energy to a small number of eggs and promoting a high survival rate of those eggs by brooding embryos through to late stage planulae (Rinkevich and Loya, 1979a). The small number of offspring per brood is offset by having many broods a year. This compares to

broadcasting species which spawn over a brief period (Kojis and Quinn, 1981b; Szmant, 1986; Van Veghal and Kahmann, 1994b). However, over-generalisations of the relationship between coral life-history strategies and morphology to reproductive mode should be avoided, as many coral species do not strictly follow all these characteristics. For example, the large colonies of a dominant Pacific reef building coral, *Acropora palifera*, brood planula larvae (Kojis, 1986a), and polyp and colony size are not related across all species of *Porites* in the Pacific (Harriott, 1983a). The number of exceptions to the rule led Fadlallah (1983a) to surmise that there is no universal relationship between colony size and morphology and reproductive mode. Fadlallah and Pearse (1982a) suggested that, for some species, the mode of reproduction directly relates to the skeletal design of the polyp corallum. For example, the large skeletal chambers in the polyps of *Balanophyllia elegans* are able to accommodate the relatively large planulae of this species. Van Moorsel (1983) also related the number of gametes and planulae produced to polyp structure, using parameters such as the number and arrangement of the mesenteries. A greater number of mesenteries per polyp will allow for a higher density of gametes and provide the capacity for many small larvae rather then a few large ones.

Examining those species that exhibit variations in reproductive mode with geographic location advances the discussion regarding the relative importance of the factors selecting for the reproductive mode of a coral species. As more research is conducted on the same species from different locations, it appears that aspects of reproductive strategies are both species and location specific. The Pacific scleractinian species *Pocillipora damicornis* has been widely researched, and there is reproductive information on this species across its distribution. Colonies of *P. damicornis* brood planulae in the Central and West-Pacific (Richmond and Jokiel, 1984; Richmond, 1985; Jokiel *et al.*, 1985; Richmond, 1987) and the Great Barrier Reef (Harriott, 1983). In these areas, the colonies are out-competed by overgrowth and shading of the dominant acroporids. The disturbance caused by competition and high adult mortality in these areas is believed to favour the production of brooded planulae to replenish the population (Richmond, 1987). At Rottnest Island in Western Australia, *P. damicornis* has been shown to exhibit broadcast spawning by some colonies in addition to planula production, although the origin of the planulae is believed to be asexual (Ward, 1992). Colonies inhabiting areas exposed to the greatest disturbance brooded a higher

proportion of larvae compared to populations living in areas of low disturbance that were mainly broadcast spawners. In the Eastern Pacific, this species reproduces by broadcast spawning (Glynn *et al.*, 1991). In contrast to the Central and West-Pacific, competition and adult mortality is low and the fast growth rate of *P. damicornis* enables it to become the dominant species (Richmond, 1987). Many populations propagate asexually in this location, but those colonies that have invested in sexual reproduction reproduce by a broadcasting mode. The reduced energy allocation to sexual reproduction and the delay in first reproduction allow for increased colony growth and dominance.

Further examples of species that adopt a different reproductive mode at geographically separated populations are not always associated with obvious environmental discrepancies. Populations of *Pocillopora verrucosa* brood planulae at Enewetak Atoll in the Central Pacific, and this is thought to aid retention of propagules in the shallow water habitat (Stimson, 1978). However, the same species in the Red Sea is a broadcast spawner even though the habitat is similar at this higher latitude reef (Shlesinger and Loya, 1985) and off the coast of South Africa (Kruger and Schleyer, 1998). Another species with a changeable reproductive mode is *Acropora humilis*, which is documented as brooding planulae in the Central Pacific and spawning gametes in the Red Sea and the Great Barrier Reef (Richmond and Hunter, 1990). Finally, an example of a coral exhibiting both sexual reproductive modes at once is *Goniastrea aspera*. Members of a population in Okinawa release gametes for external development and then retain a portion of eggs for sexual production of planulae (Sakai, 1997). Other populations of this species studied from the Great Barrier Reef are broadcast spawners (Babcock, 1984; Harrison *et al.*, 1984; Willis *et al.*, 1985). Coral reproductive mode is therefore not always consistent within a species and thorough studies on individual species are needed across geographic locations.

1.3 Coral sexual pattern and sex ratios

Within each reproductive mode, colonies can be either hermaphroditic or gonochoric, leading to four basic types of sexual pattern for corals: hermaphroditism combined with either broadcasting or brooding, and gonochorism combined with either broadcasting or brooding (Szmant, 1986). The dominant reproductive pattern recorded in scleractinian corals is hermaphroditic broadcast spawning, with gonochoric broadcast spawning adopted by more species than hermaphroditic brooding or gonochoric brooding (Harrison and Wallace, 1990). Regarding sexuality alone, hermaphroditism has been reported in 68% of scleractinian species studied by 1990 (Richmond and Hunter, 1990). In comparison, all gorgonian species studied to date are gonochoric (Chapter 4). The majority of alcyonaceans are also gonochoric, with only three species recorded as hermaphroditic (Benayahu *et al.*, 1990). Sexuality in the zoanthids is divided, with a few exceptions, along the suborders: species belonging to Macrocnemia are gonochoric, and those within the Brachycnemia are hermaphroditic (Ryland, 1997). The majority (~87%) of the studied anemones are primarily gonochoric (Table 6.1 in Shick, 1991). Scleractinian sexuality is also fairly consistent within taxonomic lines (Harrison, 1985). In the Red Sea, hermaphroditism and gonochorism follows family and even genus lines (Shlesinger *et al.*, 1998). Other families exhibit differences in sexuality between geographic locations, notably between the Pacific and the Caribbean populations, probably the result of evolutionary divergences (Harrison, 1985; Szmant, 1986). On the Great Barrier Reef, for example, the Faviidae species studied were mostly hermaphroditic (Kojis and Quinn, 1982) whereas the faviid *Montastrea cavernosa* in the Caribbean is gonochoric (Szmant, 1986). Gonochorism is generally the rule in the family Poritidae in the Indo-Pacific (Kojis and Quinn, 1981a; Harriott, 1983a), in contrast to the dominance of hermaphroditism in the Caribbean Poritidae (Szmant, 1986; Soong, 1991). However, (Szmant, 1986) concluded that there are no universal trends in reproductive mode (i.e. broadcasting versus brooding), and consequently, even though sexuality trends tend to follow taxonomic lines, overall sexual patterns in corals are systematically unpredictable.

Many hermaphroditic coral species have male and female gametes that develop simultaneously, a requirement for a mass spawning event, thereby creating the potential

for self-fertilisation. Laboratory studies have shown that some species are able to self-fertilise (Kojis and Quinn, 1981b), although cross-fertilisation is normally favoured and generally produces a higher percentage of developing embryos (Heyward and Babcock, 1986). Selfing is thought to be an adaptation to promote fertilisation in relatively uncommon, sparsely distributed species (Kojis and Quinn, 1981b; Kojis and Quinn, 1982; Heyward and Babcock, 1986). The spawning of hermaphroditic broadcasters is commonly well synchronised within a population, which is clearly an effort to promote cross-fertilisation (Kojis and Quinn, 1982; Babcock *et al.*, 1986; Heyward and Babcock, 1986). Many species release eggs and sperm packaged in buoyant gamete bundles where the sperm bundles remain isolated until reaching the surface, and this delay before being motile promotes mixing with other coral spawn (Babcock, 1984; Harrison *et al.*, 1984; Heyward and Babcock, 1986; Szmant *et al.*, 1997). Delayed receptivity of eggs has also been reported to further reduce selfing (Babcock and Heyward, 1986; Coll *et al.*, 1994; Hagman *et al.*, 1998a). Furthermore, there is a correlation between sexuality and sperm type in scleractinian corals, with hermaphroditic species having pear-shaped and ovoid sperm compared to the conical shaped sperm of gonochoric species (Harrison, 1985; Harrison, 1990). The former shape is indicative of a greater swimming potential which may be an adaptation to promote cross-fertilisation. It is also assumed that there are mechanisms to avoid hybridisation during mass spawning events, such as self-recognition (Heyward and Babcock, 1986) and the use of sperm attractants (Coll *et al.*, 1994). However, recent genetic evidence has shown that mass spawning may actually be a reproductive strategy to promote hybridisation in some taxa (Willis *et al.*, 1997; Hatta *et al.*, 1999) in accordance with the theory of reticulate evolution (Veron, 1995).

The proportional allocation of resources to male and female gametes may also reflect adaptations to cross-fertilisation. Some hermaphroditic brooders produce vast quantities of sperm, above the density required if selfing was predominant (Szmant, 1986). A further adaptation to ensure cross-fertilisation is sequential development of eggs and sperm, as occurs in the brooding hermaphrodite *Stylophora pistillata* (Rinkevich and Loya, 1979b). A variation of sexual pattern is the occurrence of gynodioecy in a population (Dunn, 1974), which is the absence of male colonies and the presence of hermaphroditic and female colonies. This has been recorded for *Porites astreoides* in Jamaica (Chornesky and Peters, 1987), and may allow both cross and self-fertilisation,

as discussed in Chapter 3. Another form of gynodioecy has been recorded for *Galaxea fascicularis* in the Pacific. Populations have both female and hermaphroditic colonies, although the eggs of the hermaphroditic colonies are non-viable and solely serve as a flotation device to bring the sperm to the surface during spawning to facilitate mixing (Harrison, 1988).

Many gonochoric broadcasting species allocate a similar reproductive effort to both male and female gamete production (Szmant, 1991). For example, *Montastrea cavernosa* has an even sex ratio at Puerto Rico (Szmant, 1986), Panama (Soong, 1991) and Colombia (Acosta and Zea, 1997), and this consistency over varying environmental conditions indicates a genetic control of colony sex (Acosta and Zea, 1997). Skewed sex ratios occurring in populations of some species have been attributed to a high incidence of asexual reproduction by fragmentation leading to the proliferation of one genotype. Examples of this phenomenon are populations of *Porites andrewsi* at Heron Island (Kojis and Quinn, 1981a) and *P. furcata* in Panama (Soong, 1991). A biased sex ratio in a population may, in some species, be an adaptation to promote fertilisation. For example, fertilisation rates in the gorgonian *Briareum asbestinum* are low and there is a positive relationship between embryo production and nearby male densities, so it is beneficial if populations are male dominated (Brazeau and Lasker, 1990). Alternatively, skewed sex ratios may also represent the greater energy and space allocation needed for brooding by female colonies. The sex ratio of the Caribbean coral *Siderastrea radians*, which is a gonochoric brooder, is biased towards female colonies (Szmant, 1986; Soong, 1991). This is in comparison to its congener *S. siderea*, that is a gonochoric broadcaster with an even sex ratio (Soong, 1991). Since only the female colonies of *S. radians* brood planulae, for which space is limiting, a higher proportion of female colonies in the population is more reproductively efficient and also avoids potential sperm wastage (Szmant, 1986). Not all observations support this proposition however, as reports of other gonochoric brooding species, such as *Balanophyllia elegans* (Fadlallah and Pearse, 1982a) and *Porites porites* (Tomascik and Sander, 1987) show them to have an even sex ratio.

1.4 Variation in reproductive effort

Reproductive effort, or fecundity, is frequently expressed as the number or volume of eggs, spermaries or planulae per polyp. A more precise measure that is occasionally used is a comparison of the proportion of energy invested in the production of gametes as opposed to that invested in somatic tissue (Jokiel, 1985; Richmond, 1987; Coma *et al.*, 1995b; Coma *et al.* 1998). These histological measures are of potential reproductive effort, and other studies have monitored the actual reproductive output by quantifying the number or percentage of gametes or planulae shed during a spawning event (for example, Van Moorsel, 1983; Szmant-Froelich *et al.*, 1985; Tomascik and Sander, 1987; McGuire, 1998). Fecundity estimates based on egg or spermary production alone must be interpreted with caution, since this may not be representative of the number of propagules produced (Szmant-Froelich *et al.*, 1985; Brazeau and Lasker, 1990). Another method is to measure reproductive efficiency by examining fertilisation success through embryo and larval development. This has been measured for various gorgonians (Brazeau and Lasker, 1992; Lasker and Stewart, 1992; Lasker *et al.*, 1996a; Coma and Lasker, 1997a; Coma and Lasker, 1997b) and scleractinian species (Heyward and Babcock, 1986).

The measured reproductive effort of corals often varies among and within colonies. Commonly, differential allocation of energy to reproduction is associated with colony age, and also size and location within a colony. Juvenile stages of life cycles are poor competitors on account of their inferior size (Roff, 1992), and so will be vulnerable to mortality by such factors as overgrowth, storm damage or sedimentation. Resources are therefore initially allocated to growth and there is a delay before the onset of reproduction until a certain age or size is reached (Connell, 1973; Kojis and Quinn, 1981b; Szmant, 1986). For scleractinian species, the calculated age at first reproduction varies between reproductive modes (section 1.2). For brooding species, it is commonly 1.5-2 years (Stimson, 1978; Rinkevich and Loya, 1979a; Szmant-Froelich *et al.*, 1985) to 2-3 years (Loya, 1976; Kojis, 1986a). Broadcasting species invest a greater number of juvenile years to growth, and the delay before reproductive activity is in the range 4-5 years (Harriott, 1983a; Wallace, 1985; Dai *et al.*, 1992; Fan and Dai, 1995; Fan and Dai, 1998) through to 5-7 years (Szmant, 1986; Babcock, 1988). Gorgonian broadcasting

species also delay first reproduction for several years in order to reach a suitable size for the optimum production of gametes (Grigg, 1979; Wahle, 1983a; Brazeau and Lasker, 1989; Kapela and Lasker, 1999; Beiring and Lasker, 2000). In contrast to the scleractinians, however, reproduction is similarly delayed for those gorgonian species with a brooding reproductive mode. As examples, *Muricea fruticosa* and *M. californica* are sexually mature after five and ten years respectively (Grigg, 1979). The externally brooding *Paramuricea clavata* in the Mediterranean has an extended period of 13 (and up to 19) years before reproductive activity (Coma *et al.*, 1995b). This species has a relatively slow growth rate and so the late age at first reproduction allows time for sufficient growth in height to 11-30 cm. The externally brooding *Briareum asbestinum* achieves a comparatively small maximum height, and consequently reaches reproductive maturity after only 2-3 years (Brazeau and Lasker, 1990).

Colony size and age are associated within a species, being affected by growth rate. The importance of colony size rather than age in regulating the direction of energy towards either growth or reproduction is shown after fragmentation occurs, such as for colonies of *Montastrea annularis* which were no longer reproductive if fragmented below a certain size (Szmant-Froelich, 1985). Similarly, previously reproductive adult colonies of the gorgonian *Plexaura homomalla* reduced in height to <20 cm by fragmentation or injury no longer produced gametes (Wahle, 1983a). However, fecundity of *Porites astreoides* was determined to be a function only of colony age (skeletal thickness of the colony) and not size (Chornesky and Peters, 1987).

Once a colony has attained reproductive age or size, there may be an initial differential allocation of energy to the production of male and female gametes. The production of male gametes is a less costly process that is a more viable option for a small colony that still needs to invest energy in growth (Charnov, 1982). Several hermaphroditic species of the family Faviidae have been shown to exhibit adolescent protandric hermaphroditism (Kojis and Quinn, 1981b; Harriott, 1983a; Kojis and Quinn, 1985), as does the brooding species *Stylophora pistillata* (Rinkevich and Loya, 1979a). Similarly, in the gonochoric externally brooding gorgonian *Briareum asbestinum*, male colonies become sexually reproductive at a smaller size than the females (Brazeau and Lasker, 1990). The reduction in energy investment in growth as a coral colony increases in size is sometimes correlated with a proportionately greater fecundity per

unit area of some scleractinian and gorgonian species (Rinkevich and Loya, 1979a; Kojis and Quinn, 1981b; Babcock, 1984; Babcock, 1988; Van Veghal and Kahmann, 1994b; Coma *et al.*, 1995b; Beiring and Lasker, 2000). This relationship between colony size and fecundity is not always linear, however, the decrease in oocyte fecundity among the largest colonies (>200 cm) of the gorgonian *Pseudoplexaura porosa* being an example of a non-linear relationship (Kapela and Lasker, 1999).

The density of gametes and planulae may vary with position within a coral colony and this has to be considered when collecting samples for studies of fecundity. The polyps at the actively growing tips of the scleractinian branching corals *Stylophora pistillata* (Rinkevich and Loya, 1979a) and some *Acropora* species (Wallace, 1985) are sterile, as all energy is diverted to growth. The 'young' polyps immediately behind the sterile growing zone were found to be less fecund than older polyps (Wallace, 1985). The growing edges of some boulder or mound shaped colonies similarly have a lower fecundity than the colony centres, as in *Porites astreoides* (Chornesky and Peters, 1987), *Montastrea annularis* (Van Veghal and Kahmann, 1994b) and *Goniastrea aspera* (Sakai, 1998). However, the scleractinian *Goniastrea favulus* showed no variation in fecundity between the polyps at the centre or the colony edges (Kojis and Quinn, 1981b). Colony growth in this species was shown to be uniform over the colony surface with no specific growth edge, thus allowing the even distribution of energy to reproduction. Polyps involved in competitive interactions will also have reduced fecundity, as has been shown for *Montastrea annularis* (Szmant, 1991). Gamete density similarly diminishes towards the branch tip of the gorgonians *Plexaura kuna* (Brazeau and Lasker, 1989), *Briareum asbestinum* (Brazeau and Lasker, 1990) and *Paramuricea clavata* (Coma *et al.*, 1995b). However, there are no intra-colony differences seen in *Pseudoplexaura porosa* (Kapela and Lasker, 1999) or *Plexaura kuna* (Beiring and Lasker, 2000). The polyps from the distal areas of the gorgonian *Paramuricea clavata* actually had a higher fecundity than the inner branch areas (Coma *et al.*, 1995a; Coma *et al.*, 1995b). Allocation of energy to reproduction is therefore a trade-off between directing resources to growth, defence and repair or to reproduction (Roff, 1992) and fecundity is commonly not uniform with a coral colony.

Spatial variation in the reproductive effort of the same species within a location is also a concern when sampling for reproductive studies. Inter-colony variation in the times of

maximal development of gametes, and their release, varies within the populations of some broadcasting gorgonian species causing peak spawning to occur in different months for separate colonies at the same location (Brazeau and Lasker, 1989; Kapela and Lasker, 1999; Beiring and Lasker, 2000). Fecundity has also been shown to vary within *Acropora* species in the same location (Wallace, 1985), and Johnson (1992) found that a proportion of the *Manicinia areolata* population he studied had very high fecundity in comparison to other colonies with no morphological differences. At a larger scale, spatial variation in fecundity between geographic locations can often be explained by different environmental conditions, as discussed in the next section.

1.5 Effects of environmental factors on coral reproduction

Reproduction in corals, as in all animals, is under the control of both endogenous and exogenous factors. Internal, or endogenous, mechanisms in coral species are poorly understood and warrant more research. Hormonal control of spawning is indicated in the scleractinian *Montipora verrucosa*, as increased estrogen levels have been documented prior to spawning (Tarrant *et al.*, 1999) and in the water during a mass spawning event (Atkinson and Atkinson, 1992). In contrast to the paucity of knowledge regarding internal regulation, the ability of corals to respond to external cues has been widely documented. Environmental parameters act as both proximate controls, co-ordinating and regulating the reproductive cycle, and also as ultimate causes exerting selective pressure on the timing and duration of reproduction so that breeding occurs at times when it is most likely to be successful (Clark, 1979; Giese, 1987). The principal environmental factors controlling the reproductive cycle in marine invertebrates, particularly corals, are seawater temperature, photoperiod (day length), lunar cycles and tidal amplitude (reviews by Orton, 1920; Fox, 1923; Giese, 1959; Giese, 1987; Harrison and Wallace, 1990). The following is an account of the effects of these environmental factors primarily on scleractinian reproductive cycles, while the limited studies on the environmental control of gorgonian reproduction are discussed in Chapters 4 and 5.

1.5.1 Temperature

The broadcast spawning of gametes is seasonal in scleractinian corals and normally occurs over just one or two months, at a defined species specific temperature, or change in temperature, as documented for many other benthic marine animals (Orton, 1920). Gametogenic cycles are correspondingly co-ordinated by temperature changes. For several species, spawning has been reported to occur as seawater temperatures are rising (Fadlallah, 1985; Kojis, 1986b; Dai *et al.*, 1992); other species will spawn over the maximum seawater temperature (Szmant-Froelich *et al.*, 1980; Stoddart, 1985; Dai *et al.*, 1992; Van Veghal, 1994a; Fan and Dai, 1995; Van Woesik, 1995; Acosta and Zea, 1997; Wilson and Harrison, 1997), or as temperatures are declining (Tranter *et al.*, 1982; Soong, 1991; Fan and Dai, 1998). The timing of spawning in response to a particular temperature cue, and also lunar day (section 1.5.2), is often shared between different local species leading to mass synchronous spawning, as occurs for over 130 species on the Great Barrier Reef (Willis *et al.*, 1985; Oliver *et al.*, 1988) and over 102 species on the Western Australia reefs (Simpson, 1991). Synchronous mass spawning within a species enhances fertilisation and promotes outcrossing, and it is essential for fertilisation success of gonochoric and self-infertile hermaphrodites. Multi-species mass spawning events imply that the timing is associated with a common environmental advantage for the species involved. Propagule survival may also be increased as a result of predator satiation (Richmond, 1997).

Many variations to the timing of mass spawning between locations have been shown in some instances to reflect geographic differences in temperature profiles, thereby substantiating the dependence of reproductive processes on seawater temperature. The offshore reefs of the central Great Barrier Reef (GBR) are cooler than, and reach maximum seawater temperatures after, the inshore reefs of similar latitude. Spawning of the same scleractinian coral species is delayed by one lunar month on the offshore reefs (Harrison *et al.*, 1984; Willis *et al.*, 1985). Similarly, the mass spawning event at the high latitude Solitary Islands in Eastern Australia occurs 2-5 months after spawning on the GBR, coinciding with the lag in the timing of maximum seawater temperature (Wilson and Harrison, 1997). In Taiwan, the delay in the annual seawater temperature maximum between Northern and Southern Taiwan causes spawning in the majority of

the scleractinian corals in the North to occur an expected 1-2 months later than at the lower latitude (Dai *et al.*, 1992). Synchronous multi-species broadcast spawning has also been reported at a few locations in the Caribbean (Chapter 7). Spawning occurred over one or two months at the time of, and just after the maximum seawater temperature (between July and September) in Bermuda (32°N, Wyers *et al.*, 1991), the Flower Garden Banks in the Gulf of Mexico (27°N, Gittings *et al.*, 1992), in Puerto Rico (18°N, Steiner, 1995), and in Panama (9°N, Soong, 1991). Spawning of some species on the reefs of Curaçao (12°N) and in the Caribbean Sea off Colombia (11°N) occurs slightly later, in September and October, in conjunction with the delayed annual maximum seawater temperature at these locations (Van Veghal, 1993; Van Veghal, 1994a; Acosta and Zea, 1997).

The synchrony of spawning among different species also varies geographically, with a breakdown in synchrony occurring with decreasing latitude from the south to the north of the GBR in association with the narrowing temperature range (Kojis, 1986b; Oliver *et al.*, 1988). The month of spawning is not as precisely defined by temperature in equatorial regions, as there are longer periods of the year for which conditions are favourable for breeding. Kenyon (1995) observed a similar pattern of decreasing synchrony of spawning among different species moving towards lower latitudes in the Central Pacific, as compared to the multi-species synchrony of the Southern GBR. Similarly, there is minimal synchrony of spawning in the Red Sea, where environmental conditions and seawater temperature are moderately stable (Shlesinger and Loya, 1985), compared to the Arabian Gulf that experiences seasonal temperature cycles (Fadlallah and Lindo, 1988). Synchronous spawning, however, does still occur in some equatorial areas, such as reported from the Solomon Islands (8°S, Baird *et al.*, 2001) and in Singapore (1°N, Guest *et al.*, 2002), implying an overlying selective advantage of synchrony and possibly the importance of other proximal cues.

Exceptions to the rule of seawater temperature being the determining factor in the timing of spawning arise when other proximate and ultimate cues play a more important role. Mass spawning of corals occurs in the austral autumn in Western Australia (Simpson 1991) and in the spring on the Great Barrier Reef (Willis *et al.*, 1985; Oliver *et al.*, 1988). The annual seawater temperature profiles along Western Australia and the

Great Barrier Reef are similar, suggesting that temperature is not the primary factor determining the reproductive season. Instead, mass spawning at each location coincides with the annual strengthening of south-bound currents, a selective advantage enhancing dispersal of the planulae away from equatorial regions (Simpson, 1991).

The ultimate evolutionary factors selecting spawning to occur outside the temperature optimum can also be associated with conditions favourable to the survival of the larvae (Giese, 1987). Spawning of *Goniastrea aspersa* takes place in the same months (October and November) at Magnetic Island on the Great Barrier Reef (19°S) as at Palau (7°N), rather than following the corresponding seasonal temperature profiles at the different locations (Babcock, 1984). The dominant environmental factor in these cases is the tidal cycle, which follows a similar regime at each location at this time of year. Spawning occurs when the low water of spring tides falls during the night, avoiding the exposure of newly settled larvae to sun stress. In North Taiwan, some species spawn one or two months out of synchrony with the mass spawning events (Dai *et al.*, 1992; Fan and Dai, 1998). Rather than coinciding with rising or maximum temperatures, these spawning times occurred after the seasonal disturbances of typhoons and heavy rainfall, when there is improved larval survival post-disturbance and increased availability of substratum for settlement (Dai *et al.*, 1992; Fan and Dai, 1998).

Changes in daylength (photoperiod) have been shown to be a spawning cue for many marine invertebrates (Giese, 1959; Giese, 1987). Seasonal change in daylength is a product of latitude similar to temperature range, and for some species it is not clear whether gametogenesis and spawning is initiated by increasing daylength or rising seawater temperature over the summer period, or a synergistic effect of the two parameters (Kruger and Schleyer, 1998). Babcock (1984) showed the importance of photoperiod alone to the timing of the reproductive cycle of *Goniastrea aspersa* by causing an alteration of spawning times with experimental modification of daylength. Photoperiod is also thought to be the proximate factor controlling spawning at the high latitude reefs of the Albrolhos Archipelago in Western Australia, where mass spawning occurs at the same time as on the tropical reefs of Western Australia (Babcock *et al.*, 1994). The temperature maximum occurs two months later in the Albrolhos Archipelago, whereas seasonal variations in daylength patterns are similar at the two

locations. Under conditions of mixed environmental parameters, the cues co-ordinating the reproductive cycles of broadcasting corals will be those that are the strongest and most significant, and this may be species-specific and geographically variable.

Variations in environmental parameters can similarly affect the timing of the breeding season of brooding corals, which generally takes place over a longer season than the gamete release of broadcasting species (Fadlallah, 1983a; Harrison and Wallace, 1990; Richmond, 1997). Study on brooding coral species has not been as extensive as the research on the more abundant broadcasting species, and the factors controlling the brooding reproductive cycle are not as widely documented. One of the most extensively studied coral species is *Pocillopora damicornis*, which has a wide distribution in the Pacific. This species, although a broadcast spawner in the Eastern Pacific (Glynn *et al.*, 1991, section 1.2), reproduces sexually by brooding across the rest of the Pacific (Harriott, 1983; Richmond and Jokiel, 1984; Richmond, 1985; Richmond, 1987). Differences in the duration of the reproductive season of *P. damicornis* occur between separated populations in concordance with the different local temperature regimes. In equatorial and low latitude regions, where temperature fluctuations are minimal, *P. damicornis* planulates year round (at Palau 7°N, Yamazato *et al.*, 1991; and Enewetak atoll 12°N, Stimson, 1978; Richmond 1987). At higher latitude reefs, reproduction is inhibited by the cooler winter seawater temperatures and release of planulae occurs seasonally over the period of favourable temperatures (at Japan 26°N, Yamazato *et al.*, 1991; Hayashibara *et al.*, 1993; and Heron Island, GBR 23°S, Stoddart, 1985). Reproduction can also be inhibited by high summer temperatures, such as the population of *P. damicornis* at Lizard Island, GBR (14.5°S) where release of planulae occurs primarily in the winter months and there is only limited activity in the hot summer period (Harriott, 1983).

Another brooding coral species that has been studied across geographic locations is *Stylophora pistillata*. Like *P. damicornis*, there is spatial differentiation in the duration of planulation in this species. Year-round release of planulae occurs in the equatorial region of Palau (Yamazato *et al.*, 1991). At higher latitude reefs, the breeding season of *S. pistillata* is restricted to the summer periods (at Okinawa, Yamazato *et al.*, 1991; and at Heron Island, Tanner, 1996). *S. pistillata* has also been well studied from the Red

Sea and the Arabian Gulf, which are both high latitude reefs but experience different environmental conditions that affect the reproductive season. The Arabian Gulf (27°N) has a wide temperature range and the release of planulae occurs outside of the temperature extreme, in the spring time when the seawater temperature is rising (Fadlallah and Lindo, 1988). The central Red Sea (24°N) has a moderate temperature range, and release of planulae can occur year round in the absence of stressful temperatures (Fadlallah and Lindo, 1988). At Eilat, further north in the Red Sea (29.5°N), the range in temperature is similar to the range in the central Red Sea but absolute temperatures are slightly cooler. The release of planulae is therefore seasonal, and is restricted to an extended period in the summer (Rinkevich and Loya, 1979b).

The duration of planulation has been documented for only a few brooding species in the Caribbean, and these data are mainly from studies in separate geographic locations. Information is available for *Porites astreoides* from four geographic locations, and this species follows the trend seen in the Pacific with an increasing duration of planula release with decreasing latitude in association with a narrowing temperature range (see chapter 5). The reproductive season of many Caribbean brooding corals is not synchronised among species within a geographic location, and this intrinsic variation requires further research before ultimate selective forces can be assigned, as their influences are clearly species-specific (discussed in Chapter 7).

1.5.2 Lunar periodicity

Subsequent to the environmental co-ordination of the month of spawning for broadcasting species, the actual day, or days, of spawning and/or final gamete maturation is synchronised by the lunar cycle (see review by Harrison and Wallace, 1990, for scleractinian species and Brazeau and Lasker, 1989; Lasker *et al.*, 1996a, for gorgonian species). Spawning at a specific lunar period has the associated ultimate cue of tidal amplitude changes. Spawning on neap tides between the full and new moons, when currents are least, is presumed to enhance fertilisation by the mixing of gametes near the parent population (Babcock *et al.*, 1986). The majority of broadcast species spawn specifically at dusk or at night and so there is additional control by the diel light

cycle (Kojis and Quinn, 1982; Harrison *et al.*, 1984; Shlesinger and Loya, 1985; Babcock *et al.*, 1986; Szmant, 1986; Brazeau and Lasker, 1989; Lasker *et al.*, 1996a). The ultimate cause for night time release of gametes is that predation will be reduced and the gametes will be less susceptible to sun stress during mixing at the water surface (Babcock, *et al.*, 1986). Release of planulae also often occurs at night for similar reasons (Stoddart, 1985; Szmant-Froelich *et al.*, 1985a; McGuire, 1998; Vermeij *et al.*, 2003).

The planulation of some brooding species does not follow a lunar rhythm, and the continuous release of planulae implies a constant cycle of gametogenesis (Rinkevich and Loya, 1979b; Harriott, 1983; Tomascik and Sander, 1987). Other brooding species exhibit a weak lunar periodicity with planulation over several days of the reproductive month indicating that either the release of planulae or gametogenesis, or both, is under limited control (Van Moorsel, 1983; Szmant-Froelich *et al.*, 1985; Kojis, 1986a; Tanner, 1996; McGuire, 1998). In contrast, a strict lunar periodicity similar to that associated with spawning in broadcasting species, is exhibited by *Manicinia areolata*, which releases most planulae within just two days of the new moon (Johnson, 1992). This species also has a short reproductive season, with planulae being released over just two months of the year. This high synchrony will increase survival of the larvae through predator satiation, similar to the effect achieved by mass spawning by broadcasting species, and predation will be lower on the dark nights of the new moon (Johnson, 1992). The moon phase at which peak planulation occurs in other brooding corals is often species specific: around the first quarter and full moon for *Favia fragum* (Szmant-Froelich *et al.*, 1985; Szmant, 1986; Soong, 1991), and for an extended period over the new moon for *Porites astreoides* (McGuire, 1998; chapter 3). Lunar periodicity to planulation can also vary spatially, for example between geographic populations of *Pocillopora damicornis* (Jokiel, 1985; Tanner, 1996), and even varies within the same populations (Harriott, 1983; Jokiel, 1985a; Jokiel *et al.*, 1985).

1.5.3 Effects of stress on coral reproduction

Under periods of stress or changes in typical environmental factors, coral reproductive cycles and reproductive effort may be altered as there will be a greater need for

allocation of energy to growth and maintenance instead of reproduction (Kojis and Quinn, 1985). Temperature anomalies cause stress to corals and can create annual differences in the timing of spawning or depress reproductive effort, and this is discussed in Chapter 5. Salinity variations can similarly affect corals, such as after heavy local rainfall or increased land run-off. Coral colonies can often withstand salinity changes for a period of time, but gametes and larvae are relatively stenohaline (Richmond, 1996; Richmond, 1997). For example, the mass spawning event at Magnetic Island in the GBR in 1981 coincided with heavy rainfall subjecting the coral spawn to lethal reduced salinity levels and a high proportion were destroyed (Harrison *et al.*, 1984). In addition, Richmond (1996) demonstrated that a drop in salinity of 20% from ambient caused an 86% decrease in fertilisation rate in the study coral species. Temperature and salinity stress, as well as changes in many other variables, often show a synergistic stress effect to corals lowering the environmental tolerance to any one variable (Coles and Jokiel, 1978).

Depth is an environmental parameter combining changes in light, water movement and in some places temperature and salinity. Increasing depth has been correlated with a decrease in fecundity as conditions fall below optimum (Stimson, 1978; Karlson, 1981; Kojis and Quinn, 1983; Kojis and Quinn, 1984; Wallace, 1985; Rinkevich and Loya, 1987). Stress thresholds will vary considerably between species and other studies have found no relationship between fecundity and depth (Richmond, 1987). Sedimentation, with its concomitant turbidity, may also be a function of depth as wave energy causes mixing of benthic sediments in shallow water and many coastal reefs are additionally affected by land run-off (Richmond, 1993). Corals subjected to sedimentation will concentrate energy to cleaning resulting in reduced growth and reproduction (Kojis and Quinn, 1983; Fadlallah, 1985; Tomascik and Sander, 1987; Van Veghal and Kahmann, 1994b). Sediment can also be a barrier to settling planulae (Loya, 1976; Richmond, 1997). Run off and sedimentation can further effect coastal corals through eutrophication (nutrient enrichment), especially from sewage and industrial outfalls. Exposure to elevated nutrients has been shown to compromise gamete size in some *Acropora* species (Ward and Harrison, 2000). Egg size of *Montipora capitata* similarly was decreased after exposure to ammonium, and planulation of *Pocillopora damicornis* ceased after four months of ammonium enrichment (Cox and Ward, 2002). Elevated levels of nitrogen and phosphorus also compromised fertilisation success in several

scleractinian corals (Harrison and Ward, 2001). Other corals may not suffer directly from increased nutrient levels, although may become overgrown by the enhanced growth of reef competitors such as algae, sponges, tunicates and bryozoans (Birkeland, 1977; Goreau *et al.*, 1981; Tomascik, 1991; Richmond, 1997). Other water pollutants, for example oil contamination (Loya and Rinkevich, 1979; Loya and Rinkevich, 1980; Guzman and Holst, 1993) and anti-fouling paints (R. Owen, pers. comm.) can cause detrimental affects to coral growth and reproduction. Increasing anthropogenic and environmental changes to coral reef areas are likely to cause further examples of stress effects on coral reproduction, which will become apparent as research continues.

Chapter 2: The sub-tropical environment of Bermuda

2.1 The Bermuda platform

The Bermuda platform is an elliptical atoll-like formation situated in the North Atlantic at 32° 20′N latitude and 65° 50′W longitude (Figure 2.1). The platform has a total area of 775 km^2, the majority of which is submerged, except for the Bermuda islands which lie at the South and Southeast of the platform and cover 50 km^2 (Morris *et al.*, 1977). The structure of the Bermuda platform is different from that of a true atoll reef in the Pacific and this led Verrill (1900) to propose the term "pseudoatoll" for Bermuda. Pacific atolls typically have a ring of small low-lying islands with an exposed rim to the platform that rapidly slopes to deep water without the development of a wide terrace reef. In comparison, the Bermuda islands are large in size and height (reaching 79 m) and are the only exposed parts of the platform, the remainder of the rim being submerged. Seaward of the platform rim there is a wide area of shoaling reef before the sea floor slopes away to the Atlantic basin. The formation of the Bermuda platform began with a volcanic eruption 40 to 30 million years ago creating the Bermuda seamount, which has since subsided and is now at an average depth of 75 m below sea level. The overlying platform present today is depositional in origin, formed from accumulations of aeolian (wind blown) limestone lithified into dunes. This sandy limestone cap was created predominantly during the Pleistocene age (10,000 – 1,600,000 bp) when changes in sea level alternately exposed and submerged the Bermuda seamount (Verrill, 1906; Stanley and Swift, 1968; Rowe, 1998). During the later Holocene rise of sea level, upward coral growth covered the Pleistocene reefs in shallow water leaving the reef formations present today (Kuhn *et al.*, 1981; Logan, 1988).

A relict feature of the Pleistocene is the Rim Reef that encircles the platform. This band of reef was once an exposed shoal of sediment which has subsequently drowned (Garrett and Scoffin, 1977; Tomascik and Logan, 1990; Logan and Tomascik, 1991). The present Rim Reef is a shallow (2-6 m), annular coral reef tract constructed from the

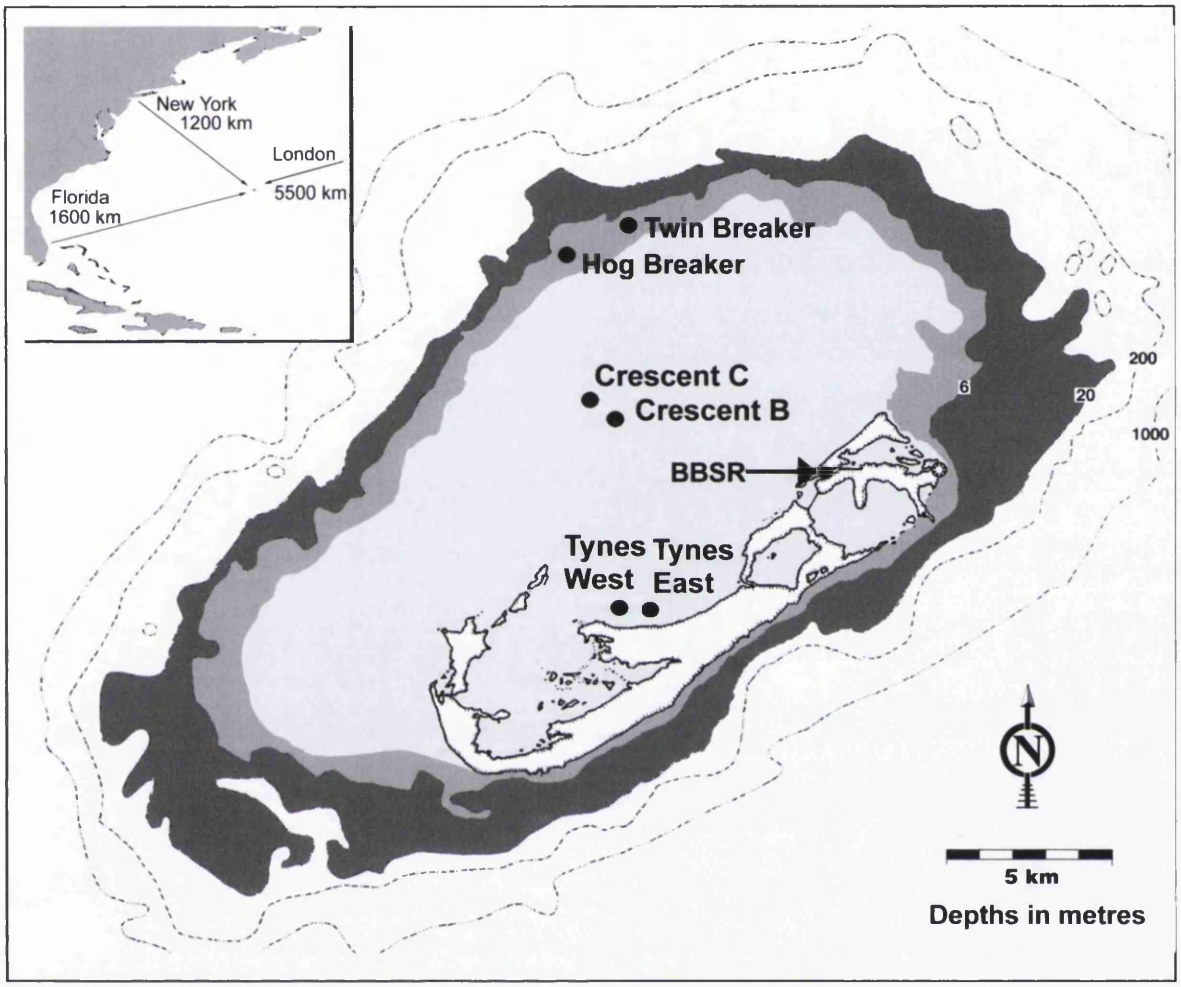


Figure 2.1: Map of Bermuda showing the Atlantic location (insert) and the reef zones of the Bermuda platform: main Terrace (dark grey), Rim Reef (medium grey) and North Lagoon (light grey). The North Lagoon can be divided further into the Inner Lagoon, close to shore, and the Outer Lagoon. Replicate study sites were set up at the two zones of the North Lagoon and at the Rim Reef. BBSR is the Bermuda Biological Station for Research.

two major reef-building consortia in Bermuda: the coral-algal reefs, and the crustose coralline algae and vermatid gastropod communities known as boiler reefs or algal-vermatid reefs (Logan, 1988). Many sand-filled channels dissect the Rim reef and form gullies with an average depth of 10 m. The Rim Reef varies from 0.5-1 km wide, shelving away seaward to the Terrace Reef, which supports an active coral-algal reef growth at an average depth of 18 m and width of up to 6 km in places (Garrett and Scoffin, 1977). Inshore of the Rim Reef is the enclosed shallow lagoon known as the North Lagoon, as it lies to the North and Northwest of the islands. The depth of the lagoon is variable, being an average of 18 m in the many depressions but shoaling to less than 1 m from the sea surface along numerous ridges. There are thousands of isolated patch reefs that occur in the Lagoon, an estimated 25,000 >5 m in diameter (T. Murdoch, pers. comm.). These are roughly circular, vary in diameter up to 800 m, and are at a depth of 18 m to 1-4 m from the surface (Scoffin, 1972). Their evolution is originally from a low coral mound forming on the sea floor at a shallow depth which then grew upwards and laterally (Wilson, 1969). The Bermuda platform can therefore be geographically divided into three zones, namely the patch reefs of the North Lagoon (which can be further divided to the Inner Lagoon and Outer Lagoon (see section 2.2)), the outer Rim Reef, and the main Terrace. The coral populations and environmental conditions defining these reef zones are discussed in the next sections.

2.2 Coral populations of the Bermuda reef zones

A decrease in the number of coral species and overall coral coverage has been reported for high latitude reefs by many authors (Kinsman, 1964; Grigg, 1981; Crossland, 1988; Harriott *et al.*, 1994; and Verrill, 1906 for Bermuda). Of the 72 known Caribbean scleractinian coral species, only 26 have been recorded in Bermuda (Sterrer, 1986). Notable absences to the island are the important reef-building corals of the genus *Acropora* found in the Caribbean. Of the approximately 50 reef dwelling gorgonian species in the Caribbean (Cairns, 1977), 23 species are present in Bermuda (Sterrer, 1986). The impoverished coral fauna in Bermuda has classically been attributed to the direct stress of low winter seawater temperatures and distance from the Caribbean limiting recruitment (Verrill, 1906). The low seawater temperature and reduced daily

solar irradiance at higher latitudes slows coral calcification rates and tissue growth (Crossland, 1981, 1984; Grigg, 1981; Tomascik and Logan, 1990 in Bermuda; Logan and Tomascik, 1991, in Bermuda). Higher latitude also favours increased competitive ability by macroalgae, which can proliferate with higher nutrient levels and reduced grazing pressure (Wiebe *et al.*, 1981; Johannes *et al.*, 1983). However, some high latitude reefs, although lacking in coral species numbers, have high coral coverage (Harriott *et al.*, 1994), and this is the case in Bermuda.

Trends similar to decreasing coral growth rates with latitude have been shown with increasing depth within a reef area, associated with diminishing light levels (Stoddart, 1969; Loya, 1972; Davies, 1980; Hudson, 1981). In Bermuda, the highest growth rates of *Porites astreoides* and *Diploria labyrinthiformis* correspondingly occur inshore and decline towards the edge of the platform as depth increases (Dodge and Vaisnys, 1977; Logan and Tomascik, 1991). It has been proposed that this pattern is caused by lower wave energy inshore and increased productivity and associated food availability (from Beers and Herman, 1969; Morris *et al.*, 1977). Fricke (1985) recorded hermatypic coral growth to 50-70 m at the Terrace Reef in Bermuda, this being a shallower depth limit than the Caribbean, where he documented coral growth as continuing to around 100 m. This may be caused by increased competition with macroalgae at depth in Bermuda and a decreased availability of suitable substrata.

The most densely populated reefs of the Bermuda platform are on the main Terrace with 30 to 60% coral cover (Smith *et al.*, 1984; Logan, 1988). The dominant scleractinian coral species in descending order are *Diploria strigosa*, *D. labyrinthiformis*, *Porites astreoides*, *Montastraea franksi*, and *M. cavernosa*. Coral coverage at the Rim Reef and on the lagoonal patch reefs ranges from 16–38% (Smith *et al.*, 1984). The species assemblage on the Rim Reef is the same as on the main Terrace; in contrast, the different physiographic conditions of the patch reefs cause them to be distinct. Species diversity is greatest on the patch reefs, with the number of species increasing with decreasing wave exposure, i.e. towards the inshore reefs and enclosed basins (Ginsburg and Stanley, 1970; Garrett and Scoffin, 1977; Dodge *et al.*, 1982). The patch reef communities are dominated by *Montastraea franksi*, *Madracis decactis* and *Porites astreoides*, and the hydrozoan *Millepora alcicornis* (Smith *et al.*, 1984). Localised dense areas of *Madracis mirabilis* and *Oculina diffusa* occur, especially on the reef

slope and face where these branching species can better tolerate the high sedimentation rates of inshore reefs (Wilson, 1969; Smith *et al.*, 1984). There are no detectable patterns of gorgonian species density and abundance recorded within or between the Bermuda reef zones (Smith *et al.*, 1984). The growth rates of some gorgonian species have not been documented to vary with depth (Mitchell *et al.*, 1993), although growth form changes are described to occur under different energy conditions in the Caribbean (Brazeau and Lasker, 1988). Colony forms are often bushy at shallow, wave exposed sites but with increasing depth branch lengths increase and the number of branches per colony decreases. Correspondingly, in Bermuda the colonies growing in the lower energy environment of the shallow inshore patch reefs are taller than the colonies offshore that are shorter and bushier (Smith *et al.*, 1984; Chapter 4). From a survey in Bermuda, the sea rods *Pseudoplexaura* spp. dominated 16 out of 22 reef sites, with the subdominant species being the sea feather *Pseudopterogorgia* spp. and the sea rod *Plexaura flexuosa* (Smith *et al.*, 1984). Other common gorgonian species are the sea rods *Plexaura homomalla* and *Eunicea* spp. and the sea fan *Gorgonia ventilina*.

2.3 The Bermuda environment

The Bermuda islands differ from many coral reef areas in that they lie outside the trade wind belt and are therefore associated with variable wind direction (Morris *et al.*, 1977). In the summer the wind comes predominantly from the Southeast to South-Southwest at an average of less than 15 knots (Smith, 1998). In the autumn the wind direction is commonly from the East-Northeast to North and in winter South-west to West winds predominate, often greater than 15 knots, and gales (>35 knots) are common from the Northwest (Smith, 1998). Therefore, the North Lagoon is often sheltered in the summer but experiences stronger autumn winds and bears the full force of the winter gales, creating high wave energy. A few tropical storms affect the island each year, creating extreme conditions, but hurricanes are less frequent (2-5 years). The outer Rim Reef is the most exposed, with wave height in the winter of 5-20 m from the long fetch of the surrounding ocean. The lagoonal reefs are protected to a degree by the outer reefs but can experience 2-4 m wave heights during gales and hurricanes.

Adequate rainfall of 147 cm per year reaches the islands of Bermuda, evenly distributed through the seasons (Smith, 1998). As the rock is porous, there is little freshwater run off and consequently, even though salinity values will decrease slightly in the inshore basins, the North Lagoon and Rim Reef water remains relatively stable at 35.5. Bermuda water reaches high clarity, in excess of 50 m, offshore at the Rim Reef during the winter. Seasonal variation does occur with an increase in turbidity in the summer and autumn to an average light attenuation of 25 m. There is a gradient of increasing turbidity moving from the Rim Reefs to inshore. Secchi readings reach a maximum of 20-25 m in the winter in the outer Lagoon and fall to 7-10 m in the summer. Turbidity increases further inshore to an average of 5-10 m visibility year round and this is partly caused by the greater levels of productivity in the water column increasing nutrient levels, for example, decomposed particulate matter, excretory products from fauna and land run-off (Beers and Herman, 1969). In addition, the inshore waters are impacted by coastal developments and also affected by the regular passing of large cruise ships that leave a plume of sediment in their wake (A. Waltham, unpub. data; S.R. Smith, unpub. data). These sediments in the shallow waters are readily re-suspended by wave energy.

2.4 Seawater temperature

Coral Reefs predominate in the tropics between 30°N and 30°S where minimum water temperatures do not fall below 18°C. Sustained temperatures below 16°C for a few weeks are detrimental to most reef-building corals (Jokiel and Coles, 1977; Roberts *et al.*, 1983), and lower temperature dips to below 15°C for just a few days have caused widespread coral mortality in some areas (Walker *et al.*, 1982; Burns, 1985). The high latitude reefs that occur outside these tropical regions are associated with warming bodies of water, such as the Kuroshio Current reaching the coral reef islands off Japan (Tribble and Randall, 1986) and the warm winter current extending to the Abrolhos Islands off Western Australia (Crossland, 1981). The islands of Bermuda owe their existence to warm water eddies from the Gulf Stream that reach the platform maintaining the winter seawater temperature above that of adjacent waters (Hela *et al.*, 1953), thereby preserving Bermuda as the most northerly coral reef in the Atlantic.

High latitude reefs experience a wide annual range in seawater temperature and temperature variation in Bermuda is greater than in the lower Caribbean (CARICOMP, 1997). The annual temperature patterns and variations that occurred in temperature profiles across the Bermuda platform were monitored throughout this study by the use of *in situ* calibrated Onset Stowaway data loggers (accuracy ±0.05°C, resolution ±0.02°C). Data loggers were deployed at the replicate sites of the reef zones of the Bermuda North Lagoon that were monitored for coral reproduction studies (the outside Terrace reefs were not included): the Inner Lagoon, Outer Lagoon and Rim Reef. Data were logged every 88 minutes and a daily average calculated.

Seawater temperature in Bermuda is coolest in the winter months (January-March) and then begins to increase in April to a summer peak over July-September (Figure 2.2). Oceanic waters buffer the Rim Reefs that therefore fall to a winter minimum of only 18 to 19°C. The Inner Lagoon is less influenced by these oceanic waters and the shallow Lagoon seawater temperature cools to below 17°C during winter with spiky dips of 15.5 to 16°C occurring, such as in March 1999 and in January 2000. Similarly, the offshore reefs largely remain at oceanic water temperature in the summer and only briefly reach temperatures in excess of 29°C. The greatest temperature range occurs at the inshore waters that rise to 30-31°C in the summer. The Outer Lagoon temperatures lie between the two extremes of the range at the inshore and offshore reefs, forming a temperature gradient across the Bermuda platform.

There was inter-annual variation in the summer seawater temperature patterns over the study period: 1998 was a relatively warm summer, 1999 recorded moderate temperatures, and 2000 was comparatively cool. Seawater temperature in 1998 was slow to rise at the Outer Lagoon and Rim Reef zones, gradually increasing during July. Temperatures finally peaked in August from all reef zones, remaining above 29°C for an extended period over the majority of August due to the lack of summer storms that year. The Rim Reef waters uncharacteristically reached a maximum of 29.8°C and the Inner Lagoon temperatures increased to a maximum of 31°C. In 1999 and 2000, the

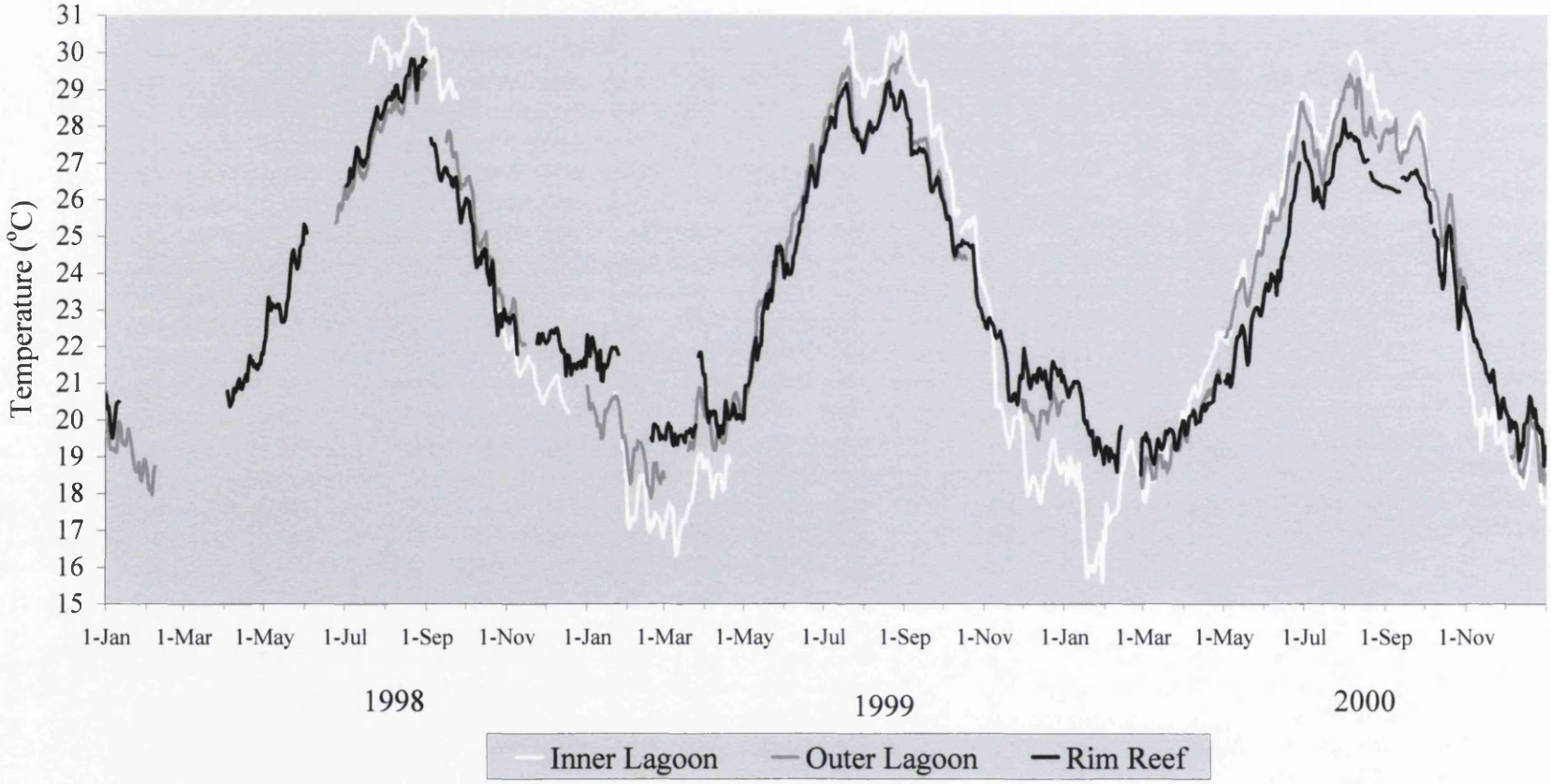


Figure 2.2: The average daily seawater temperature at the reef zones of the North Lagoon over 1998-2000.

Gaps in the data are when data loggers were not deployed, or malfunctioned.

seawater temperature rose earlier in the year than in 1998, reaching above 29°C at all the zones by July. Summer storms caused the temperatures from all reef zones to fluctuate over the summers of 1999 and 2000, which moderated the maximum temperatures. The Rim Reef and Outer Lagoon reefs in 1999 only reached temperatures above 29°C for short peaks in mid-July and August, which were separated by a cooler period. Maximum temperature from the Rim Reef was 0.7°C lower in 1999 than in 1998 at 29.1°C. The Inner Lagoon temperatures reached a maximum of 30.7°C in 1999. By comparison, 2000 was a cool summer, with the Rim Reef never reaching 29°C (max 28.2°C), and the Outer Lagoon reaching just above 29°C (max 29.3°C) for only two weeks at the end of July. The Inner Lagoon uncharacteristically did not rise above 29°C until the end of July, remaining there for just over two weeks and only attaining a maximum of 30°C for a few days. The inter-annual and inter-zone variation in seawater temperature across the Bermuda platform is discussed in relation to the timing of coral reproductive cycles and to reproductive effort in Chapter 5.

Chapter 3: Sexual Reproduction of *Porites astreoides* (Scleractinia: Poritidae) in Bermuda

3.1 Introduction

The corals of Bermuda are of particular interest to reproductive studies since the isolation and low winter seawater temperature mean that many species are at their distribution extreme (Chapter 2). Information on coral reproduction from Bermuda is limited to the work of S.C. Wyers and colleagues who studied the spawning of four broadcasting species, *Diploria strigosa, D. labyrinthiformis, Montastrea annularis* and *M. cavernosa* (Wyers, 1985; Wyers *et al.*, 1991). This study continues the documentation of coral reproduction in this sub-tropical environment by examining sexual reproduction of the mustard coral *Porites astreoides* (Scleractinia: Poritidae).

Porites astreoides (Lamarck) is a common member of scleractinian coral reef communities throughout the Caribbean (Goreau and Wells, 1967; Veron, 2000). There have been three previous studies on the gametogenic cycle of *P. astreoides* (Chornesky and Peters, 1987, in Jamaica; Szmant, 1986, in Puerto Rico; Soong, 1991 in Panama), and one documentation of the timing of the breeding season (McGuire, 1998, in the Florida Keys). All these studies have shown *P. astreoides* to exhibit a brooding reproductive mode. The gametogenic studies in the Caribbean have shown a year round presence of gametes. Planulae were found in the coral tissue throughout the year in Panama and Jamaica, with fewer present around the autumn. The production of planulae is seasonal in Puerto Rico and the northern Florida Keys. Planulae were seen in coral tissue from the Puerto Rico colonies in January until September. In the Northern Florida Keys, the release of planulae from colonies occurred primarily in April and May, with smaller numbers of planulae observed through until September (McGuire, 1998). The gametogenic studies of *P. astreoides* revealed that this species exhibits a mixed sexuality, and Chornesky and Peters (1987) recorded female and hermaphroditic colonies in the Jamaica population, whilst male, female and hermaphroditic colonies were all found in the Panama population (Soong, 1991). The

number of planula released by *P. astreoides* colonies was weakly correlated to colony area (McGuire, 1998), and Chornesky and Peters (1987) showed a positive relationship between fecundity and colony age (maximum skeletal thickness).

The relationship of *P. astreoides* planula release to the lunar cycle is also investigated. To ensure fertilisation and gamete outcrossing, many broadcasting coral species release gametes at a specific lunar phase (see review by Harrison and Wallace, 1990; Chapter 1). Several brooding coral species also show lunar periodicity to planula release (see reviews by Fadlallah, 1983a; Harrison and Wallace, 1990; Richmond and Hunter, 1990; and also Johnson, 1992; Tanner, 1996; McGuire, 1998). Other brooding species release planulae over a continuous cycle throughout the lunar period (Van Moorsel, 1983; Tomascik and Sander, 1987; Delvoye, 1988; Shlesinger *et al.*, 1998). The relationship of planulation to the lunar cycle has also been shown to vary spatially, such as for the Pacific coral *Pocillopora damicornis*, which has been studied across many geographically separate locations (Stimson, 1978; Richmond and Jokiel, 1984; Stoddart, 1985; Jokiel, 1985; Ward, 1992). The integration of the current reproductive studies on *P. astreoides* also shows synchrony to planula release to be geographically variable. The original work of Szmant (1986) in Puerto Rico did not find lunar periodicity to planulation. However, the abundance of brooded planulae in the coral tissues of colonies sampled in Jamaica peeked prior the new moon (Chornesky and Peters, 1987). Similarly, the Panama population contained planulae between the full moon and just prior to the new moon and then planulae were not found over the new moon and the first quarter moon phase (Soong, 1991). McGuire (1998) monitored the actual release of planulae from colonies, which significantly peaked over the new moon, although planulation was extended over a 21 day period. Chornesky and Peters (1987) found spermatogenesis to be under a lunar control with maturation occurring around the full moon, although Soong (1991) found spermaries of all sizes throughout the lunar cycle. The moon phase at which *Pocillopora damicornis* planula release occurs also varies within the same geographic location (Harriott, 1983; Jokiel, 1985; Jokiel *et al.*, 1985). The timing of planulation of *P. astreoides* was therefore studied at the three physiographic reef zones of the Bermuda platform that vary in environmental conditions, these most notably being temperature, wave energy and sedimentation (Chapter 2), to examine any effect of these parameters on the reproductive cycle.

Colony shape of *P. astreoides* ranges from massive to encrusting and the surface is frequently 'lumpy' or even nodular (Veron, 2000). The tentacles are normally extended during the day, capturing a variety of food from fine particulate matter to zooplankton (Lewis and Price, 1975). Many colonies of *P. astreoides* larger than 15 cm in diameter have shown evidence of splitting into several parts forming a cluster of smaller colonies (Lewis, 1974b), although this does not commonly occur in Bermuda (pers. obsv.). Genetically similar colonies of *P. astreoides* occur in two colour morphs: a green morph (yellow-green to dark green), and brown morph (light brown to dark brown; Weil, 1992). The morphs co-occur at various depths, although green colonies were shown to be significantly more abundant in shallower water than colonies of the brown morph, which related to a higher concentration of the UV filtering mycosporine-like amino acids (MAAs) in the green colonies (Gleason, 1993). Susceptibility to sedimentation was shown to differ between the colour morphs in the US Virgin Islands: brown colonies were more efficient at sediment removal compared to green morphs (Gleason, 1998) and so dominated areas of high sedimentation.

Colonies of *P. astreoides* are often covered in mucous sheets that are produced by abundant epidermal mucus gland cells in the polyps (Coffroth, 1988). These sheets are routinely observed on many poritid corals and their formation is believed to be a natural phenomenon (Bak and Elgershuizen, 1976; Ducklow and Mitchell, 1979; Coffroth, 1985; Edmunds and Davies, 1986; Kato, 1987; Coffroth, 1991). The production of excess mucus to form sheets can also be induced by stress to the colony, for example by oil-sediment coverage (Bak and Elgershuizen, 1976), salinity (Coffroth, 1985; Kato 1987) and temperature (Coffroth, 1985; Kato, 1987). Mucous sheet formation in *P. astreoides* in Panama is periodic and timed to the lunar cycle with increased formation over the first quarter moon phase (Coffroth, 1991). The presence of mucous sheets on the *P. astreoides* colonies in Bermuda is documented and examined as a possible effect on the release of planulae from the colonies.

In summary, the aspects of reproduction of the common brooding scleractinian *Porites astreoides* that have been studied are the timing and duration of the breeding season in Bermuda and the control of lunar periodicity to planula release. Patterns of gametogenesis and colony sex ratios are also examined, as is the effect of colony size on fecundity, and mucus production on planula release.

3.2 Objectives

This study addresses the following questions about the reproductive cycle of *Porites astreoides*:

1. What is the timing and duration of the reproductive season of *Porites astreoides* in Bermuda?
2. What is the sexuality of *P. astreoides* colonies in Bermuda?
3. Is there a relationship between fecundity of *P. astreoides* and colony surface area?
4. Is there lunar periodicity to the planula release of *P. astreoides* in Bermuda? Does synchrony to planulation vary between colonies at the different reef zones?
5. Is mucous sheet formation on *P. astreoides* colonies a cyclical event? Is there a relationship between the timing and number of planula released and mucous sheet formation?

3.3 Methods

3.3.1 Collection of corals and monitoring of planulation

Sampling schedule

Porites astreoides is ubiquitously distributed across the Bermuda platform on both shallow and deeper reefs (Verrill, 1906; Smith *et al.*, 1984; Sterrer, 1986; Cavaliere *et al.*, 1987; Logan, 1988). Collection sites were located within the three physiographic reef zones of the Bermuda North Lagoon (Figure 2.1, Chapter 2). A preliminary study was performed in the summer of 1998 to determine the timing of *Porites astreoides* planula release in Bermuda. Prior to the full moon (f.m.) and new moon (n.m.) periods of July and August 1998, eight colonies were collected from two study sites, one located at the Outer Lagoon (Crescent C) and the other at the Rim Reef (Hog Breaker). A further eight colonies were then sampled over the September new moon. New colonies

were collected for each sample date to minimise the possibility that stress resulting from holding the corals in aquaria might affect planulation. The colonies were held in aquaria and monitored for planula release (see sampling technique below) for four days either side of the f.m. in July 1998 (eight days in total) and from two days before the f.m. to four days after the f.m. in August 1998 (six days in total). Colony collections over the n.m. periods were made two, three and then four days respectively before each n.m. from July to September. No planulae were released from the monitored colonies over the July and August f.m. Planulation occurred over the n.m. periods from July to September 1998, and on each occasion began on the first night after collection. From this result and also the new information from McGuire (1998), all colonies were collected 7-10 days before each new moon (subject to weather conditions) in 1999 and 2000. Colonies from all reef zones were monitored in aquaria for the same time period each month. Collections were made in this way between June and September 1999 and from June to October 2000 (Table 3.1). The Inner Lagoon sites were included in 1999 and 2000. In 1999, the sample size was five colonies from each of the three reef zones in June, July and September 1999, and was increased during August 1999 to ten colonies from the Inner Lagoon and eight colonies from the Outer Lagoon and Rim Reef. The sample sizes were increased over June to October 2000 and replicate reef sites included within each reef zone. Five colonies were collected at each study site equating to ten colonies per reef zone.

Table 3.1: Sampling schedule of *Porites astreoides* colonies over the new moon periods (n.m.) of June to September 1999 and June to October 2000. Colonies were monitored in aquaria from the start date shown and including the night of the last date shown. Total days includes that of the new moon.

Month	Date of n.m.	Start	Days before n.m.	End	Days after n.m.	Total days	Sample size		
							Inner Lagoon	Outer Lagoon	Rim Reef
Jun 1999	June 14	June 6	8	June 21	7	16	5	5	5
Jul 1999	July 12	July 4	8	July 20	8	17	5	5	5
Aug 1999	Aug 11	Aug 1	10	Aug 20	9	20	10	8	8
Sep 1999	Sept 9	Sept 1	8	Sept 18	9	18	5	5	5
Jun 2000	June 2	May 26	7	June 8	6	14	10*	10*	10*
Jul 2000	July 1	June 23	8	July 11	10	19	10*	10*	10*
Aug 2000	Jul 31	July 21	10	Aug 10	10	21	10*	10*	10*
Sep 2000	Aug 29	Aug 22	8	Sept 6	8	17	10*	10*	10*
Oct 2000	Sep 27	Sep 19	8	Oct 4	7	16	10*	10*	10*

* 5 colonies were collected from each of two replicate sites within the reef zone.

Sampling technique

All colonies collected were approximately 15 cm in diameter, of the green colour morph, and had no mucus covering. The entire colony was removed from the substratum using a hammer and chisel. All corals were transported back to the laboratory in coolers of seawater kept at ambient temperature to minimise collection stress. The corals were maintained on the outdoor wet bench facility next to the dock at the Bermuda Biological Station for Research. Each colony was placed inside a 'planulae collector', which was a clear Tupperware container (2.5 litre; 18 cm x 12 cm deep) with a water flow inlet at the base to promote circulation. The planulae collectors were placed within large aquaria of flowing seawater to help maintain constant temperature. The container lids had an large window cut out which was covered in 200μm nitex mesh to allow water to flow out whilst retaining planulae within the container. The planulae of *P. astreoides* are released after sunset (McGuire, 1998; pers. obs.) so the collectors were checked each morning. All planulae were removed using a

pipette and counted. The lids to the collectors were left off during the day to encourage water flow around the colonies and reduce shading. The incidence of mucous sheet formation was also monitored for the colonies kept in aquaria over the reproductive months of July to September in 1999 and 2000. Measurements were consistently taken six days before and after the n.m. period. The percentage coverage of mucous sheet on each colony was recorded in 10% intervals. At the end of the monitoring period the surface area of the colonies was measured using the tin foil technique (Marsh, 1970). The colonies were then returned to their respective reefs and cemented in place. Subsequent observation revealed a high survival rate of the re-transplanted colonies. Reproductive effort for each colony was recorded as the total number of planula released per cm^2. The variable periods over which colonies were monitored in aquaria each month (Table 3.1) was accounted for by adjusting the measure of reproductive effort to the total number of planulae released per cm^2 per day.

3.3.2 Histological examination of reproductive structures

Tissue cores were collected from *Porites astreoides* colonies at the Outer Lagoon sites in June and July 2000 for histological examination of gametes and planulae. Four large colonies (two from each site: Crescent C and Crescent B) were tagged on June 15th (one day before the full moon) and two cores of 2 cm diameter were removed from each using a hand drill. Cores were not collected from the colony edge where reproductive polyps are less abundant (Chornesky and Peters, 1987). These colonies, along with an additional four colonies (two from each site), were sampled in the same way on June 23rd (on the last quarter moon). All eight colonies were sampled once more on July 18th (two days after the full moon).

All cores were immediately fixed in 9% seawater formalin for 36 hours before being transferred to 10% formic acid for decalcification. The formic acid was changed daily and the process was complete in 5-7 days. All tissue samples were then washed and preserved in 70% EtOH. A sharp razor blade was used to cut the tissue to a rectangle of 15 polyps that was then dehydrated, cleared and embedded in Paraplast. Serial cross sections were made at 8μm through the whole tissue from tentacles to base and the

ribbon of sections was cut after every 7 sections to fit on the slides. Every other strip of sections was mounted on slides and stained with Mallory's Triple Stain (Humason, 1962; Grimstone and Skaer, 1972). The middle section of each slide (equating to intervals of 112μm distance down the polyp) was observed under the x20 objective of a compound microscope and the number and size of all gametes and larvae within the central ten polyps recorded using the ImagePro (Version4) Image Analysis package.

3.4 Results

3.4.1 Timing and duration of planula release

Planulation was observed over the new moon periods of July and August 1998, 1999 and 2000 with a small number of planulae released in September each year. Colonies were monitored additionally over the new moons of June 1999, June 2000 and late September to early October 2000 but no planulation occurred. During the preliminary studies in 1998, colonies were collected only two, three and four days before the new moon in July to September respectively. Planulae were always released on the first night of being held in aquaria suggesting that planulation may have begun prior to collection. Measures of lunar periodicity or fecundity cannot be determined from such incomplete data and so the following results are taken from 1999 and 2000 when all colonies were monitored between 7 -10 days before and after the new moon.

3.4.2 Planula release and colony size

A total of 126 colonies from all the reef zones were monitored for planulation in July-September 1999 and 2000, and of these 93 (74%) were reproductive (not including reef zones in September 1999 and 2000 where no planula release occurred). The overall percentage of reproductive colonies from the reef zones varied between the months, ranging from 62-93% (Chapter 5). However, combining data from all months for the

three reef zones, there was little inter-zone variability in total reproductive activity with planula release occurring from 72% of the Outer Lagoon colonies, 73% of the Rim Reef colonies and 77% of the Inner Lagoon colonies. The mean (± standard deviation (SD)) surface area of all the reproductive colonies collected was 203 (±60) cm^2. The surface area of the largest colony collected was 400 cm^2, and of the smallest was 114 cm^2. Both of these colonies were reproductive. The mean surface area of the 33 non-reproductive colonies was 205 (±52.5), max 308, min 123 cm^2. One-way ANOVA was used to examine whether the colony surface area of the reproductive colonies differed from the non-reproductive colonies, and also whether colony surface area differed dependent on the zone of collection. All colony surface area data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov (Wilkinson *et al.*, 1992) and Bartlett (Sokal and Rohlf, 1995) tests respectively. Log_{10} transformation of the data was necessary to obtain normality, and the variances of the transformed data were homogeneous (Appendix 3.1). There was no significant difference between the mean surface area of the reproductive and non-reproductive colonies (P= 0.679, Appendix 3.2). Colony mean surface area did significantly differ between the three reef zones (P= 4.566×10^{-8}, Appendix 3.2) and the post-hoc test revealed that the mean surface area of colonies from all the reef zones were significantly different from each other (Appendix 3.2). Mean surface area for the Inner Lagoon colonies was 248 (max 400, min 158) cm^2, for the Outer Lagoon colonies 198 (max 332, min 118) cm^2, and for the Rim Reef colonies 175 (max 301, min 114) cm^2.

The relationship between *P. astreoides* colony surface area and fecundity was examined with correlation coefficient analysis. Log_{10} transformation of the data successfully obtained normal distributions (Appendix 3.1 for colony surface area and Appendix 3.3 for planula release data). There was no significant relationship between numbers of planula released per day and the surface areas of the reproductive colonies monitored over the study period (Figure 3.1, product-moment correlation, r= -0.033, n= 93, P= 0.755). The relationship between colony area and numbers of planula released was similarly tested for colonies collected from the different reef zones combined over the months. There was no significant relationship between the parameters from each reef (Product moment correlation, Inner Lagoon r= 0.260, n= 27, P= 0.191; Outer Lagoon r= 0.165, n= 31, P= 0.376; Rim Reef r= -0.076, n= 35, P= 0.664).

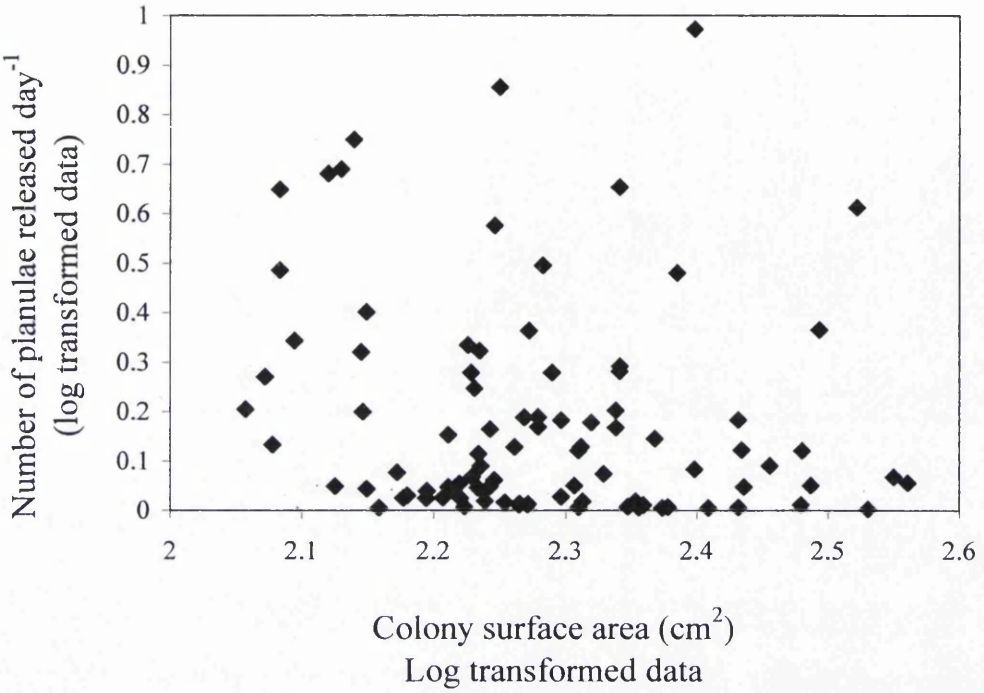


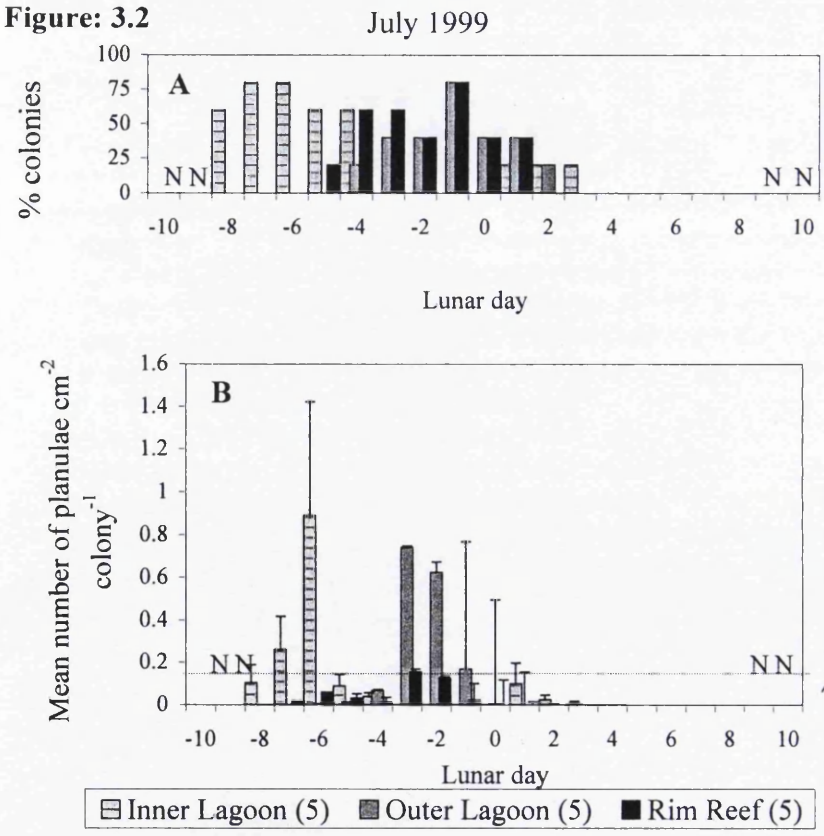
Figure 3.1 : The relationship between the number *Porites astreoides* planula released and the colony surface area. Each data point is the total number of planula released by that colony normalized by the experimental period (number of days held in aquaria, see Table 3.1 methods). Data are combined for July-September 1999 and 2000. All data were log transformed (Appendix 3.1).
N= 93 (non-reproductive colonies were not included)

The overall mean (±SD) number of planula released from the 93 reproductively active *P. astreoides* colonies monitored over each new moon period from July to September 1999 and 2000 was 0.798 (±1.386) planulae cm^{-2} of colony. The maximum number of planula released by a colony was 8.39 planulae cm^{-2} (total of 2099 planulae) and the minimum was 0.005 planulae cm^{-2} (2 planulae released). The polyp density of *P. astreoides* colonies was measured on skeletons that had been naturally dried by counting the number of skeletal cups (corallites) under a dissecting microscope, using a 1 cm x 1 cm grid. Five counts were made from the skeletons of each of five colonies. Using this method, it was estimated that the mean (±SD) polyp density of *P. astreoides* was 40.7 (±0.9) polyps cm^{-2}. McGuire (1998) measured the mean polyp density of *P. astreoides* colonies to be 61 polyps cm^{-2} of decalcified tissue, the variation likely due to the shrinkage of coral tissue upon histological processing (approximately 20-30%, Harriott, 1983a; see Table 1 in Ryland, 1997; E. Peters, pers. comm.). Conversion of the mean number of planula released per cm^2 to the number of planula released per polyp using this estimate of polyp density equates to a mean of 0.02 planulae $polyp^{-1}$ and a maximum of 0.21 planulae $polyp^{-1}$ (minimum estimates were not included as they were from the release of just two planulae per colony). Assuming that one polyp releases one planula per new moon period, the data suggest that a mean estimate of only 2% and a maximum of 21% of the polyps of *P. astreoides* colonies release planulae.

3.4.3 Inter-zone variability to lunar periodicity

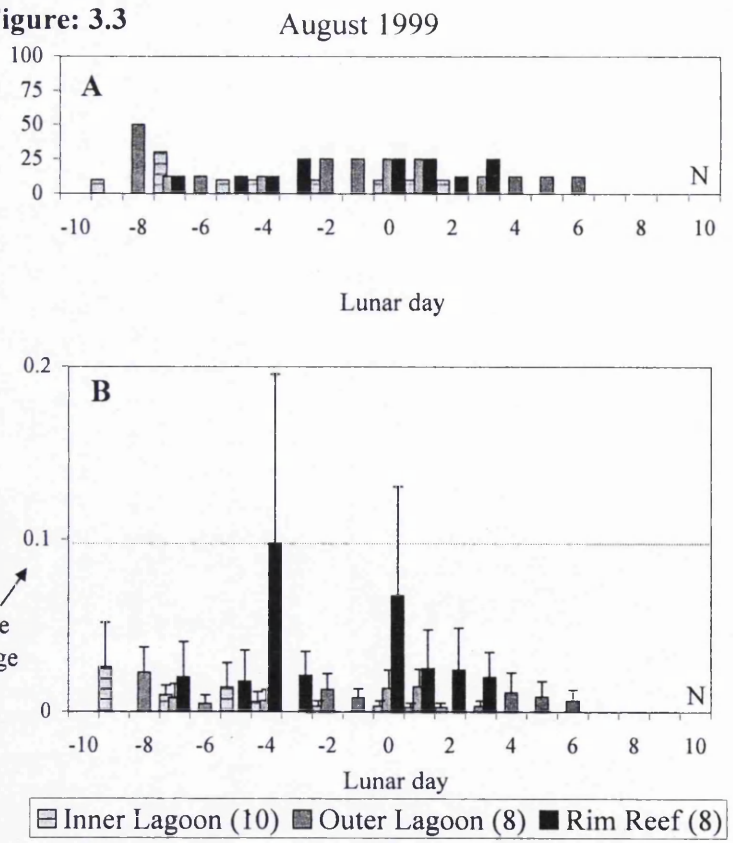
The duration of planulation from *Porites astreoides* colonies ranged from the erratic release of planulae over one to four nights to colonies that planulated for periods of up to 13 days. A few colonies planulated over two intervals that were three to four days long separated by a short inactive period. The timing of planulation varied among the reef zones as follows. In July 1999, colonies from all reef zones were monitored for planula release from eight days before the new moon (n.m.) to eight days after the n.m. Planulation occurred within the monitored time period at the Outer Lagoon and Rim Reef, lasting for an eight day period with a peak just before the n.m. (Figure 3.2). The Inner Lagoon colonies released planulae on the day of collection, eight days before the n.m. and both the percentages of colonies that planulated (Figure 3.2A) and the numbers of planula released (Figure 3.2B) peaked six days before the n.m. A second period of

Figure: 3.2



Colonies monitored 8 days before n.m. to 8 days after n.m.

Figure: 3.3



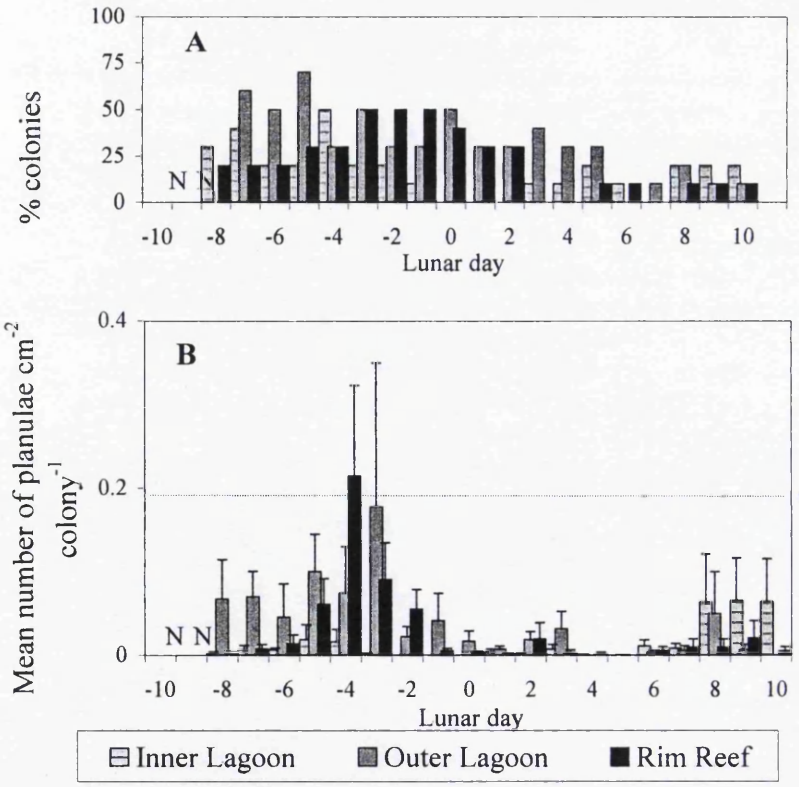
Colonies monitored 10 days before n.m. to 9 days after

Figure 3.2 and 3.3: The percentage of *Porites astreoides* colonies that planulated (A) and the mean (+1SE) number of planulae released per colony (B) from each reef zone over July 1999 (Figure 3.2) and August 1999 (Figure 3.3). N= no data. Number of colonies from each reef zone used to calculate the mean is shown in parentheses in the legend.

planula release occurred in just one colony from the Inner Lagoon for days 1-3 after the n.m. Reproductive effort in August 1999 was much lower than in July (note scale change in Figure 3.3B). All colonies planulated within the monitored period of ten days before the n.m. to nine days after the n.m. and planula release was minimal and erratic from all reef zones over the new moon period (Figure 3.3). Planula release in September 1999 occurred from just two colonies (n=5) from the Rim Reef zone for a period of 2 -3 days over the new moon period (data not graphically represented).

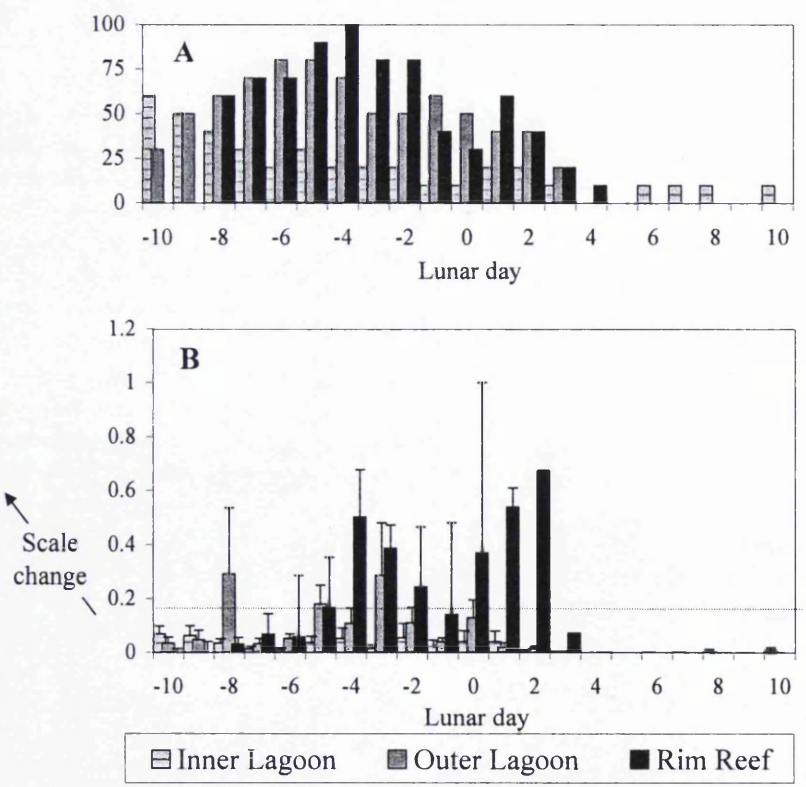
Planulae were released over extended periods in July and August 2000. A proportion of the colonies planulated throughout the monitored periods in each month (Figures 3.4 and 3.5). In July 2000, the number of planula released from the Outer Lagoon and Rim Reef peaked three and four days respectively before the n.m. (Figure 3.4B). Planulation continued thereafter intermittently from colonies collected from these reef zones until the end of the monitoring period, although this extended planulating period is primarily the result of erratic release of just a few planulae from one or two colonies (Figure 3.4A). Colonies from the Inner Lagoon released planulae from the day of collection, eight days before the n.m. in July 2000 until one day after the n.m. (Figure 3.4A). A second planulation event occurred from 3-6 days after the n.m.. The number of planulae released from these colonies was low compared to the other reef zones (Figure 3.4B). Planulation continued from two colonies (n=10) from eight days after the n.m. until the end of the monitored period and the number of planula released from these colonies was greater than over the previous 17 days. All colonies from the Inner Lagoon reef zone were therefore held in aquaria after the planned monitoring period for extended observation. Planulation only continued from the same two colonies for a further eight days and then ceased. The reproductive effort from these two colonies over this ten day period of eight days to 18 days after the July n.m. (i.e. over the July full moon) was a mean of 0.21 planulae cm^{-2} day^{-1} and 0.25 planulae cm^{-1} day^{-1}. This is greater than the reproductive effort from these same colonies over the previous July monitoring period (mean of 0.013 planulae cm^{-2} day^{-1} and 0.011 planulae cm^{-2} day^{-1} respectively). The level of reproductive effort by these aberrant colonies over the full moon phase of July 2000 is comparable to that of the Inner Lagoon colonies monitored over the August n.m period (Figure 3.5). It is believed, therefore, that these colonies were beginning the next cycle of planula release.

Figure 3.4: July 2000



Colonies monitored 8 days before n.m. to 10 days after n.m.

Figure 3.5: August 2000



Colonies monitored 10 days before n.m. to 10 days after n.m.

Figure 3.4 and 3.5: The percentage of *Porites astreoides* colonies that planulated (A) and the mean (+1SE) number of planulae released per colony (B) from each reef zone over July 2000 (Figure 3.4) and August 2000 (Figure 3.4). N= no data. The mean was calculated from 10 colonies at each reef zone.

The greatest reproductive effort occurred from the Outer Lagoon and Rim Reef zones in August 2000. Colonies from the Outer Lagoon zone planulated on the first day of monitoring, ten days before the n.m. (Figure 3.5). The percentage of planulating colonies peaked 5-6 days before the n.m. (Figure 3.5A), and the total number of planulae released from these Outer Lagoon colonies peaked eight days before the new moon and then again three days before the n.m. (Figure 3.5B). Colonies from the Rim Reef zone did not release planulae until the third night of monitoring (eight days before the n.m.). The maximum percentage of planulating colonies occurred four days before the n.m. when all colonies were reproductive (Figure 3.5A). The total number of planula released from the Rim Reef colonies peaked four days before the n.m. and then again two days after the n.m. (Figure 3.5B). Reproductive activity in the Outer Lagoon and Rim Reef colonies had ceased by five days after the n.m. The percentage of colonies that released planulae from the Inner Lagoon in August 2000 was greatest at the start of the monitoring period between 8-10 days before the n.m. (Figure 3.5A). The number of planula released from these colonies was minimal and steady from the start date until two days after the n.m. (Figure 3.5B).

Examination of the above data by combining all months and years for each reef zone demonstrates an inter-zone variation in the relationships between lunar day and the percentage of colonies releasing planulae (Figure 3.6), and between lunar day and the number of planula released from those colonies (Figure 3.7). Arc sine transformation of the data for both figures was necessary to obtain a normal distribution for regression analysis (Appendix 3.4). Planula release was not observed from any colonies collected from the Rim Reef 9-10 days before the n.m. and the percentage of colonies releasing planulae peaks just prior to the n.m. (Figure 3.6A). A percentage of the colonies collected from the Outer Lagoon over July and August 1999 and 2000 planulated throughout the monitored time in aquaria and a greater percentage of colonies were reproductively active from 4-9 days before the n.m. (Figure 3.6B). In contrast, the combined data for the Inner Lagoon shows an extended period over which colonies released planulae with a negative trend over time and an increase in the percentage of reproductively active colonies at the start and end of the monitored period (Figure 3.6C). All curves showing the relationship between the percentage of colonies releasing planulae (arc sine transformed data) and the lunar period are a significant fit to the data ($P<0.001$, third order polynomial regressions).

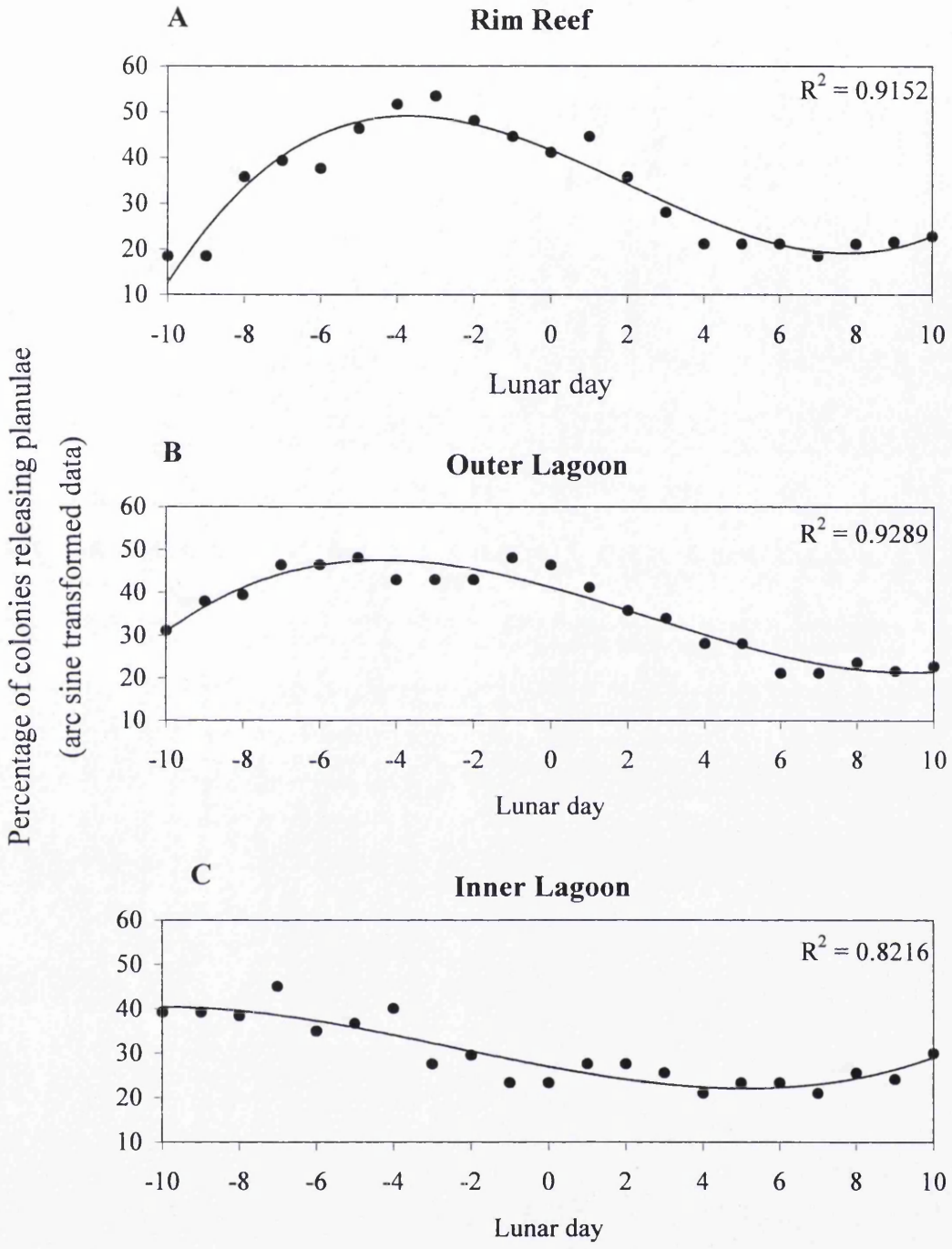


Figure 3.6: The relationship between lunar day and the percentage (arc sine transformed) of *Porites astreoides* colonies releasing planulae. Each reef zone shows all colonies combined for July and August 1999 and 2000. All curves are third order polynomial regressions ($P<0.001$). Lunar day is 10 days either side of new moon (0). Real zero values are 18.4 arc sine transformed data.

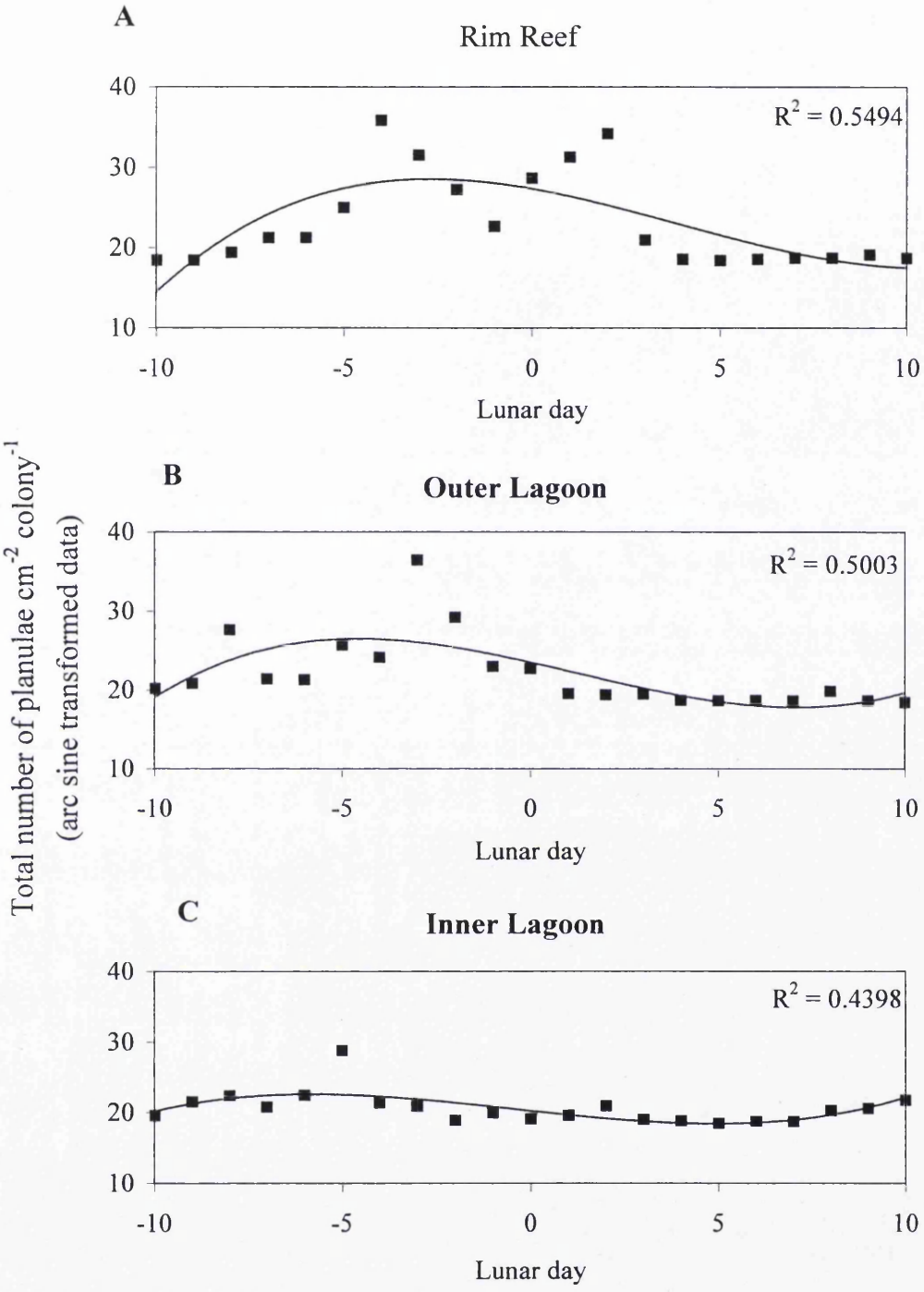


Figure 3.7: The relationship between lunar day and number of planulae released (arc sine transformed) by *Porites astreoides* colonies. Each reef zone shows all colonies combined for July and August 1999 and 2000. All curves are third order polynomial regressions ($P<0.01$). Lunar day is 10 days either side of new moon (0). Real zero values are 18.4 arc sine transformed data.

The mean number of planula released during the months of July and August 1999 and 2000 combined, and shown for each reef zone, is erratic over the monitored periods (Figure 3.7), a consequence of inter-colony variation in reproductive effort. However, the relationships between lunar day and the number of planula released for the reef zones follow a similar trend as shown for the percentage of colonies releasing planulae (Figure 3.6). The number of planula released from the Rim Reef colonies combined from July and August 1999 and 2000 peaked either side of the n.m. and diminished towards the start and end of the monitored period (Figure 3.7A). A greater number of planulae were released from the Outer Lagoon colonies prior to the n.m. (Figure 3.7B). The number of planula released from the Inner Lagoon colonies remained relatively low and constant over the monitored lunar periods (Figure 3.7C). The curved relationships between lunar day and the number of planula released (arc sine transformed data) are a significant fit to the data ($P<0.01$; all curves are third order polynomial regressions).

3.4.4 Mucous sheet formation

The formation of mucous sheets on the *Porites astreoides* colonies held in aquaria to monitor planula release was highly variable. Corals from all sites showed variability in both timing of mucous sheet formation and the degree of coverage. A proportion (27%) of colonies did not form any mucus whilst held in aquaria, or were only partially covered (<10% of the colony) for a few days before the mucus was shed. Planulation did occur from colonies that were covered in mucus and also persisted from a proportion of colonies that had over 50% of their surface area covered in mucus. Between one and thirteen planulae were recorded from colonies that had 100% mucus coverage.

Combining the data for all the colonies from each reef zone that were held in aquaria, mucous sheet formation (a colony is scored if >50% of the colony was covered in mucus) did not shown any clear reef zone-related pattern that was consistent over the months (Figure 3.8). Colonies collected in September 1999 did not form any mucus until two days before the new moon. In all other months, mucous sheets had formed on colonies from at least one of the reef zones by the start of the monitoring for mucus formation, six days either side of the new moon. In July 1999 and August 2000, the

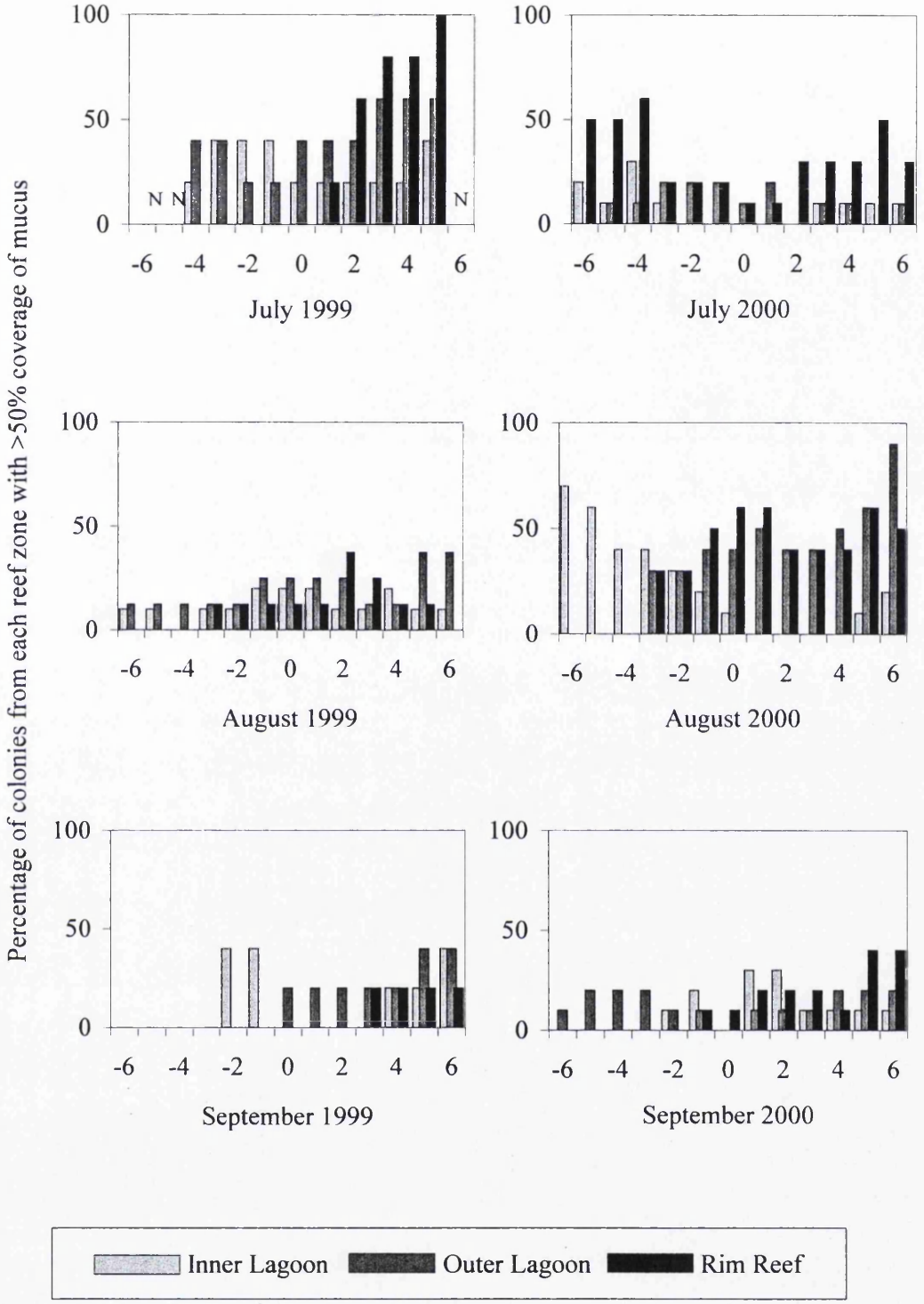


Figure 3.8: The percentage of *Porites astreoides* colonies held in aquaria forming mucus sheets over the new moon periods of July-September 1999 and 2000. X axis is 6 days either side of new moon (0). N= no data available.

greatest percentage of colonies with mucous sheets occurred towards the end of the monitored period.

To investigate the relationship between the presence of mucus and the release of planulae from colonies each month, the two parameters were plotted together for each reef zone (Figures 3.9-3.11). In July 1999, planula release from the Inner Lagoon colonies was greatest at the start of the monitored period when data on mucus was not recorded (Figure 3.9). A second peak in the number of planula released occurred when the percentage of colonies with mucous sheets fell back to 20%, which was the minimum observed over the monitored period. However, mucous sheets were never present on more than 40% of the colonies. Planulation from the Inner Lagoon colonies was erratic in August 1999, while the presence of mucous sheets was minimal and did not exceed 20% of the colonies. In the reproductive months of July and August 2000, the number of planula released from the Inner Lagoon colonies actually followed a similar trend to percent mucus presence, with the greatest reproductive activity prior to the August new moon when mucous sheets were present on 70% of the colonies (Figure 3.9). The Inner Lagoon colonies did not release planulae in September 1999 or 2000 and the percentage of colonies with mucous sheets did not exceed 40% and 30% respectively.

Over 50% of the colonies collected from the Outer Lagoon formed mucous sheets in July 1999 and August 2000 (Figure 3.10). The number of planula released was high in these months but ceased when more than 40% of the colonies possessed mucous sheets. This occurred three and four days after the n.m. for July 1999 and August 2000 respectively (Figure 3.10). In August 1999, planula release was erratic over the monitored period and there is no trend associated with the increasing number of colonies that formed mucous sheets. No planulae were released from the Outer Lagoon colonies in September 1999, even though mucous sheets did not form until two days after the new moon on only 20% of the colonies. In July and September 2000, the presence of mucous sheets on the Outer Lagoon colonies was minimal, not exceeding 20% of the colonies and showing no trend over the monitored period (Figure 3.10).

Mucous sheets formed on the Rim Reef colonies one day after the n.m. in July 1999, which coincided with a fall in the number of planula released (Figure 3.11). The

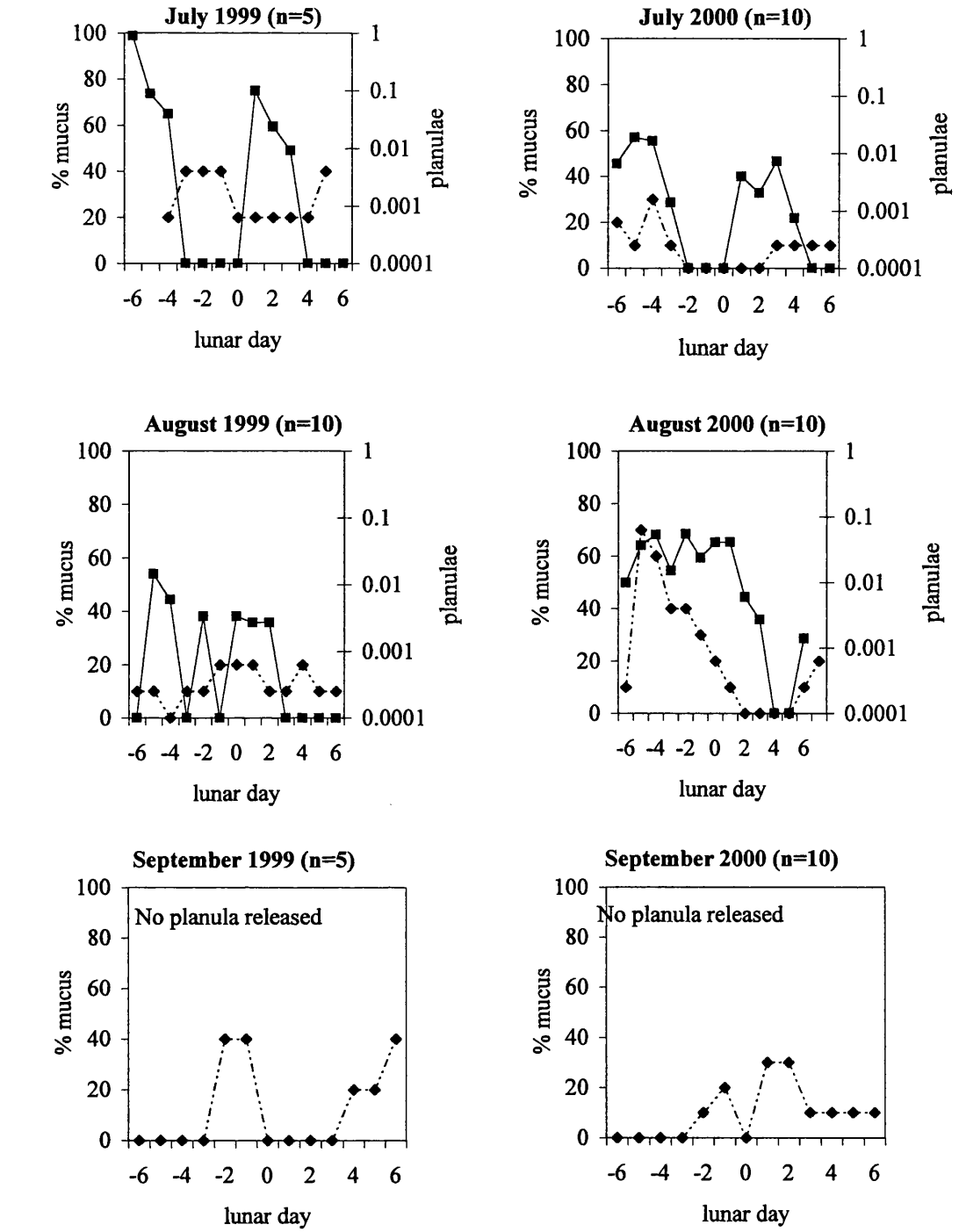


Figure 3.9 Inner Lagoon: The percentage of *Porites atreoides* colonies held in aquaria forming mucous sheets shown with the total number of planulae released for July to September 1999 and 2000 at the Inner Lagoon. Number of colonies sampled shown in brackets in the title. x axis is 6 days either side of new moon (0).

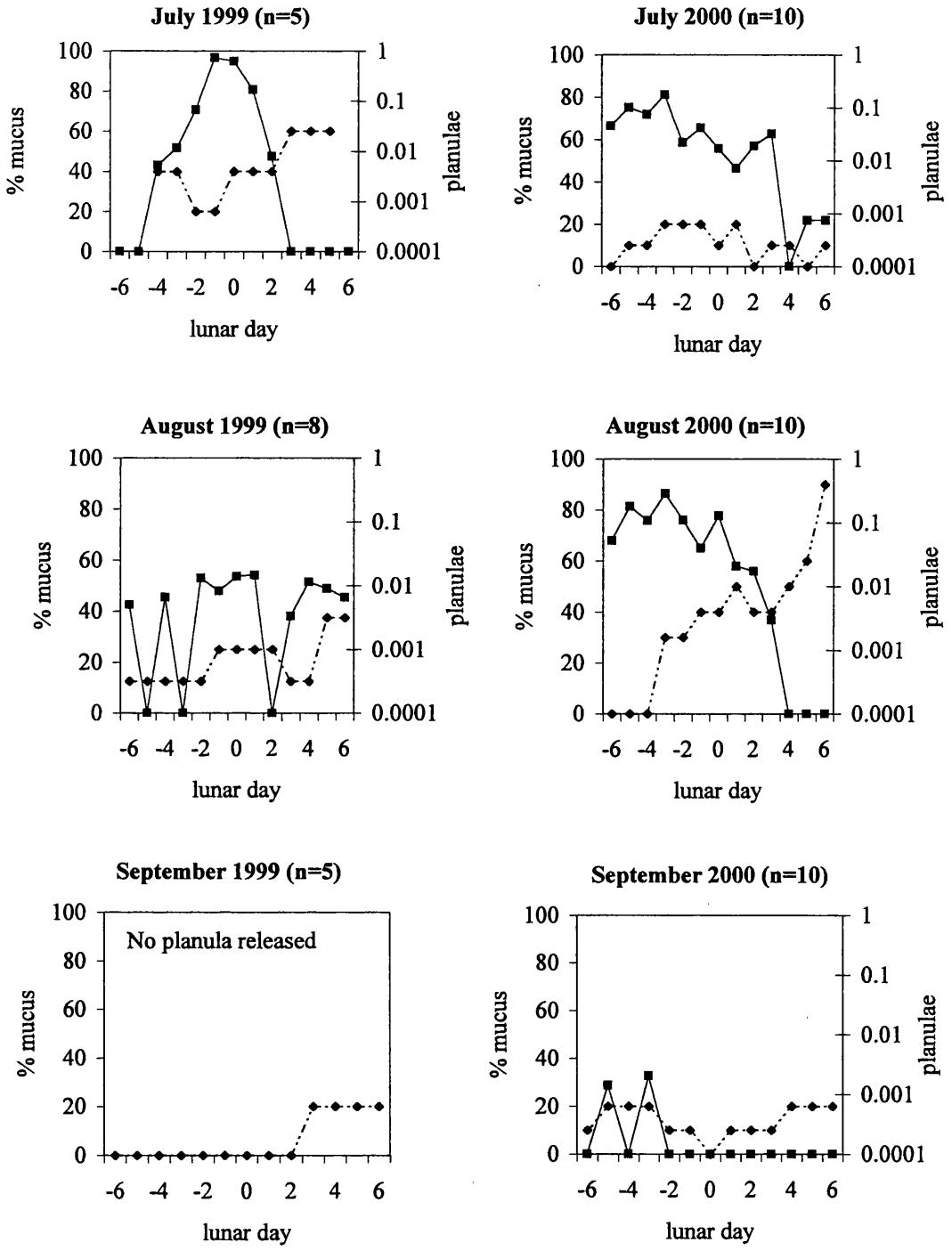


Figure 3.10 Outer Lagoon: The percentage of *Porites astreoides* colonies held in aquaria forming mucous sheets shown with the total number of planulae released for July to September 1999 and 2000 at the Outer Lagoon. Number of colonies sampled shown in brackets in the title. x axis is 6 days either side of new moon (0).

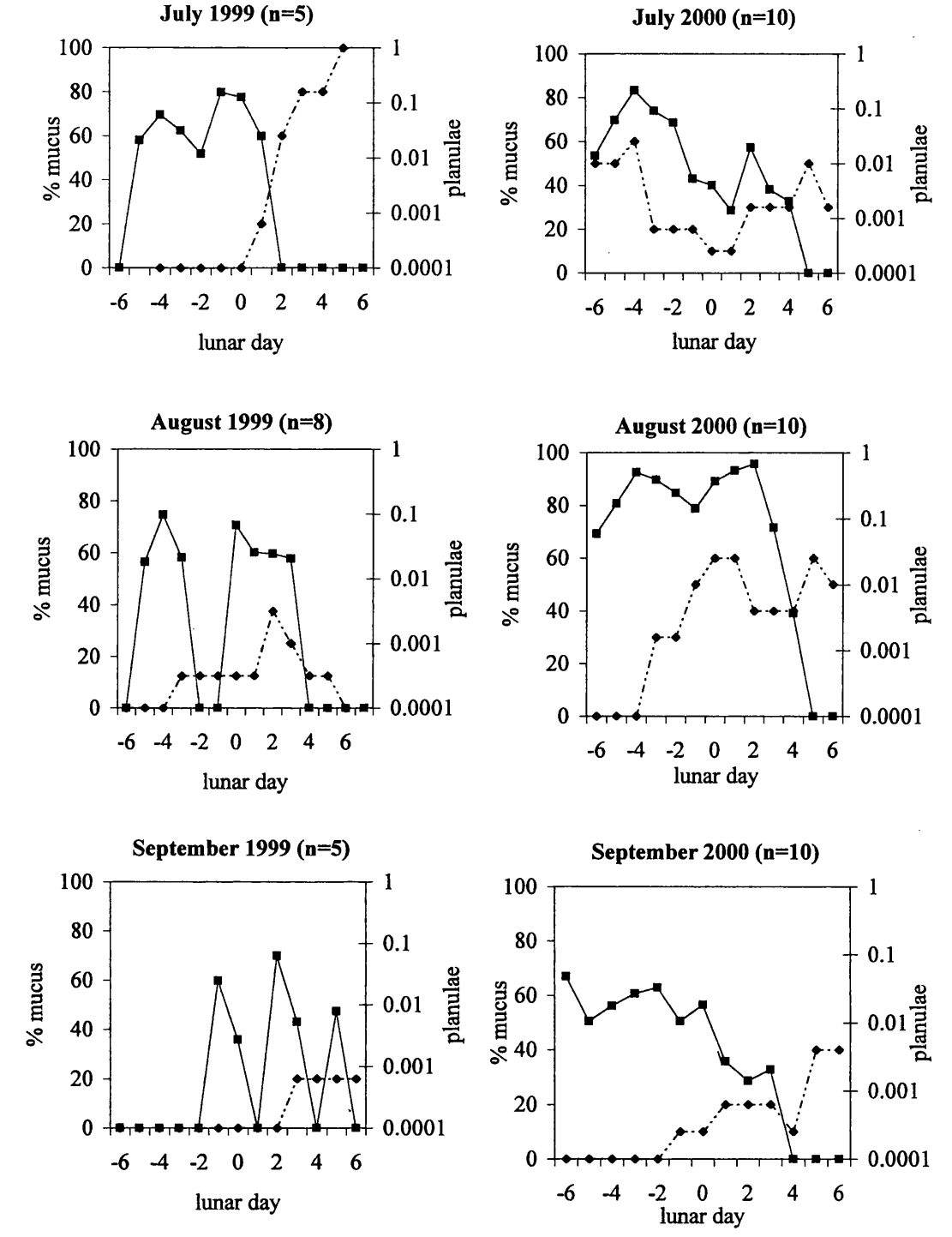


Figure 3.11 Rim Reef: The percentage of *Porites astreoides* colonies held in aquaria forming mucous sheets shown with the total number of planulae released for July to September 1999 and 2000 at the Rim Reef. Number of colonies sampled shown in brackets in the title. x axis is 6 days either side of new moon (0).

percentage of colonies with mucus increased thereafter from 20% to 40%, and planulation from all the colonies ceased. All colonies from the Rim Reef had formed mucus by five days after the n.m in July 1999. Planula release from the Rim Reef colonies in August and September 1999 fluctuated over the monitored periods, showing no relationship with the percentage of colonies forming mucous sheets (Figure 3.11). In July and August 2000, planulation continued from the Rim Reef colonies until five days after the n.m. The cessation of planula release coincided with a slight increase in mucous sheet formation, but the percentage of colonies with mucous sheets had been at a similar level earlier in the monitored period (Figure 3.11). Planula release from the Rim Reef colonies in September 2000 was at its maximum at the start of the monitored period and decreased after the n.m. as the percentage of colonies forming mucous sheets increased, although mucous sheets were only present on a maximum of 40% of the colonies.

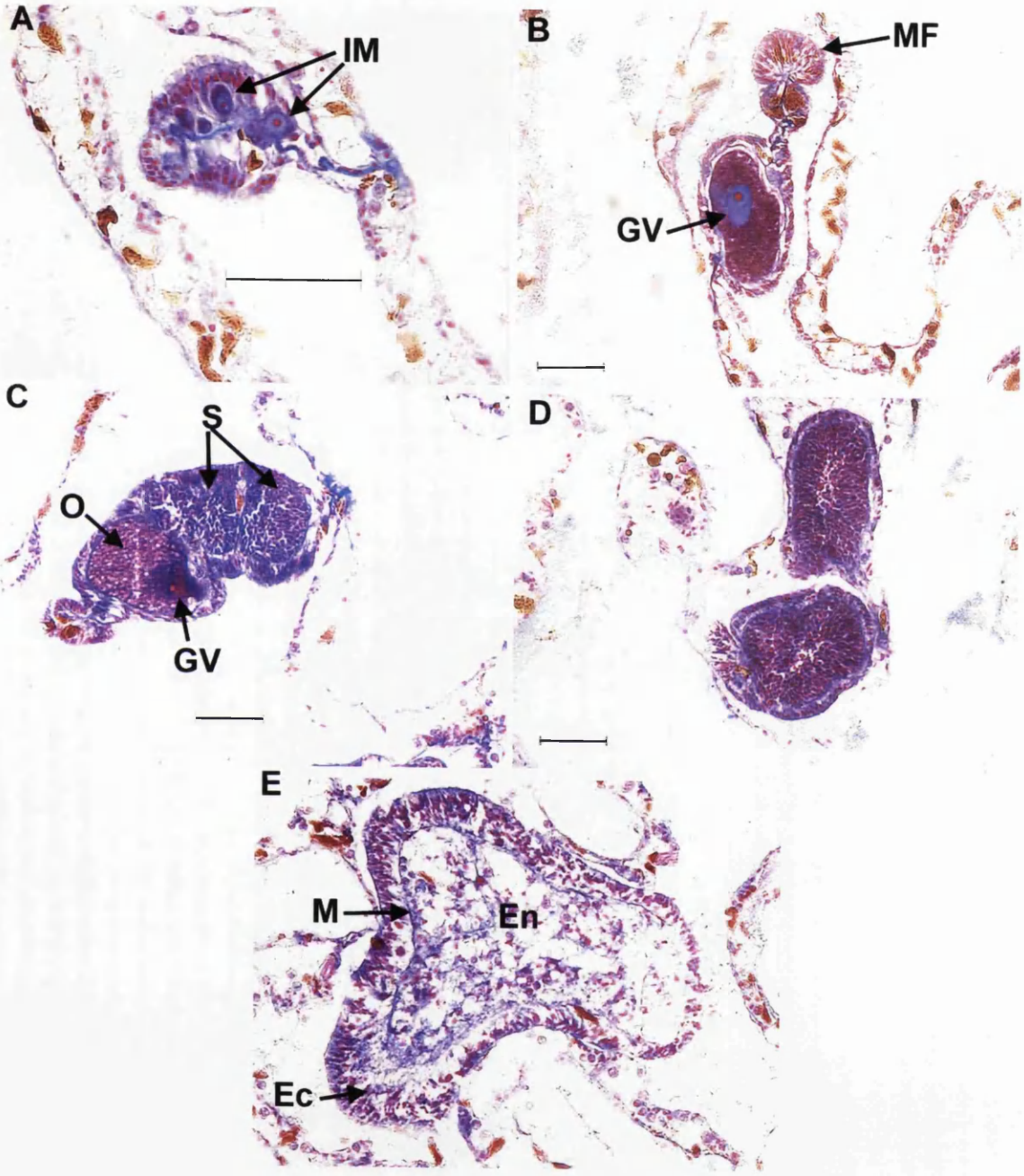
3.4.5 Colony sexuality and patterns of gametogenesis

A total of 40 cores were collected for histological examination from eight large, tagged *Porites astreoides* colonies from the Outer Lagoon reef zone. Three colonies were found to contain only oocytes, five colonies were hermaphroditic and none of the colonies contained only spermaries. Within hermaphroditic colonies, the polyps were male, female or possessed gametes of both sexes. Two planulae were found in the cores and these were from different colonies on the sample taken two days after the July f.m.

Tissue fixation and histological techniques will cause shrinkage of coral tissue by 20-30% (Harriott, 1983a; Table 1 in Ryland, 1997; E. Peters, pers. comm.), which should be considered during the following description of gametogenesis. The smallest recognisable oocyte had a diameter of 9.2 μm (IM in Figure 3.12A). The immature oocytes had a distinguishable germinal vesicle and central nucleolus surrounded by a thin layer of cytoplasm. The cytoplasm of the mature oocytes had greatly enlarged through vitellogenesis (Figure 3.12B and C). The germinal vesicle of a few large oocytes was still central (GV in Figure 3.12B). However, for most of the oocytes observed the germinal vesicle was at a peripheral position (GV in Figure 3.12C) after

Figure 3.12: Histological sections of developing oocytes, spermaries and planulae in *Porites astreoides* colonies. Tissues were fixed in 9 % formalin, decalcified with 10 % formic acid and stained with Mallory's Tripple stain.
Scale bar represents 50 μm. Tissue shrinkage of approximately 20-30 % would have occurred during the histological process.

A: Immature oocytes (IM) within mesentery
B: Mature oocyte with germinal vesicle (GV) and central nucleolus at base of mesenterial filament (MF)
C: Developing oocyte (O) and spermaries (S) within the same mesentery
D: Mature spermaries within mesentery
E: Planulae located within the coelenteron towards the polyp mouth. This is the section of maximum width. Planulae ectodermal layer (Ec) well developed, there is a thin mesoglea (M) and large endodermal cells (En) fill the interior. Mesenteries had not been formed.


A
IM
B
MF
GV
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GV
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M
En
Ec

the onset of vitellogenesis, which is characteristic of scleractinian oocytes (Rinkevich and Loya, 1979a; Szmant-Froelich *et al.*, 1985; Kruger and Schleyer, 1998). No zooxanthellae were seen in the oocytes. The maximum oocyte diameter recorded was 135.9μm. Developing oocytes and spermaries occurred either separately or were intermingled on the same mesentery (O and S in Figure 3.12C). All spermaries were of the same developmental stage over the three sample dates between the June and July full moons. It is assumed therefore that late stage spermatozoa, the tails of which are clearly visible, rapidly develop before spawning. Spermary diameter was variable, range 50-150μm, although measurements were not always at the greatest diameter as spermaries lack a central diagnostic feature, such as the nucleolus in oocytes. The spermaries were packed with spermatocytes and smaller spermatids could occasionally be seen around the small lumen (Figure 3.12C and D). The two planulae found in the slides on the July full moon sample were both located within the polyp coelenteron and had a maximum diameter of 250-300μm (Figure 3.12E). The planulae had a thick ectodermal covering (Ec), a thin mesoglea (M) and the interior was filled with large endodermal cells (E in Figure 3.12E). No mesenteries could be seen developing within the planulae. Released planulae were 1-1.5 mm long and ~0.5 mm diameter, elongate when swimming and circular when stationary (Figure 3.13A and B). The released planulae are full of zooxanthellae, which are seen as brown spots under normal light (Figure 3.13B) and glow red under fluorescent light (as Z in Figure 3.13C). The zooxanthellae are present in the endoderm (E), which is clearly differentiated in the slide squash preparations from the surrounding ectoderm (Ec in Figure 3.13C).

Figure 3.13: Slide squash sections of swimming *Porites astreoides* planulae

A: Planula larva at x4. Scale bar represents 500 μm

B: Close up of a planula larva. Scale bar represents 500 μm

C: Same planula larva under fluorescent light showing the ectodermal layer (Ec) and zooxanthellae glowing red (Z) within the endoderm (En). Scale bar represents 50 μm.

A

B

C

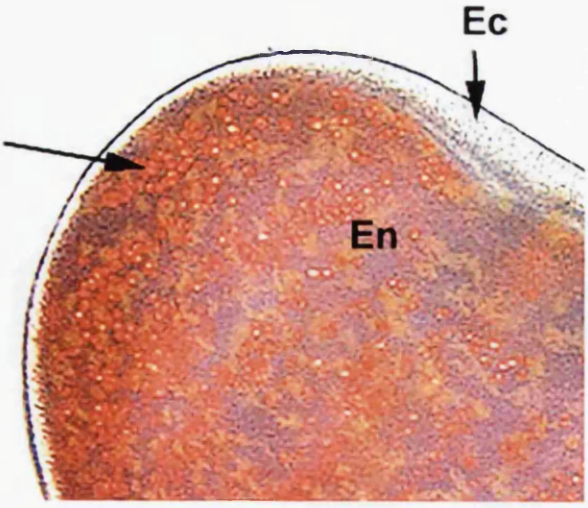


Table 3.2: Variation in the density of gametes within *Porites astreoides* colonies from the Outer Lagoon sampled around the June full moon (f.m.), the June last quarter moon and the July f.m. Gametes were counted from 20 polyps per colony on each sample date (10 polyps from each of two cores). ND= no sample collected from colony numbers 5-8 on 15-June. Colony sex: H=hermaphroditic, F=female.

		Oocytes/polyp			Spermaries/polyp		
Colony #	Sex	15-Jun-00 June f.m.	23-Jun-00 June 3/4	18-Jul-00 July f.m.	15-Jun-00 June f.m.	23-Jun-00 June 3/4	18-Jul-00 July f.m.
1	H	6.65	8.15	5.95	0	0.6	0
3	H	0.15	0.75	0.55	56.05	49	0
4	H	2.5	4.5	0.35	9.8	14.6	0
7	H	ND	2.05	0	ND	7.85	0
8	H	ND	0.2	2.85	ND	79.65	2.65
2	F	0.15	1.35	0			
5	F	ND	1.5	1.35			
6	F	ND	9.05	0			

Mean polyp fecundity varied among the colonies (Table 3.2). Combining reproductive cores from the sample dates for the three female colonies, the overall mean number of oocytes per polyp was 2.68 (max 9.05, min 0.15, n=10 reproductive cores, 100 polyps). The combined mean number of oocytes per polyp calculated from reproductive cores taken from the hermaphroditic colonies was 2.89 (max 8.15, min 0.15, n=24 reproductive cores, 240 polyps). Spermaries were overall more abundant than oocytes in the hermaphroditic colonies with a combined mean spermary density of 27.5 spermaries polyp^{-1} (max 76.65, min 0.6, n=16 cores, 160 polyps). The sex ratios of the hermaphroditic colonies were variable. The colonies with the greatest overall spermary density (colony number 3 and 8) contained the fewest oocytes and the colony with the greatest overall oocyte density (colony number 1) contained the lowest numbers of spermaries (Table 3.2). The two remaining hermaphroditic colonies (numbers 4 and 7) had a relatively low density of both spermaries and oocytes.

Spermary density from the hermaphroditic colonies increased between the June f.m. and last quarter moon phase, and then decreased to a minimum in the sample taken three

weeks later around the July f.m. (Figure 3.14), when spermaries were present in only one of the four hermaphroditic colonies (Table 3.2). The mean number of oocytes from these hermaphroditic colonies was similar between the June f.m. and last quarter moon phase, and then also declined by the July f.m. (Figure 3.14), when oocytes persisted in all but one colony (Table 3.2). Oocyte density from the female colonies was more variable than in the hermaphroditic colonies with the greatest number of oocytes present on the June last quarter sample (Figure 3.14). However, only one female colony was sampled on the June f.m., and by the July f.m. two of the three female colonies did not contain any oocytes (Table 3.2). There was no developmental pattern to the oocyte size frequency distributions to indicate a synchronised cohort growth from the limited number of samples (Appendix 3.5). A range of oocyte sizes was found on all sample dates. The greatest numbers of oocytes were of a medium size in the 40-80μm size classes.

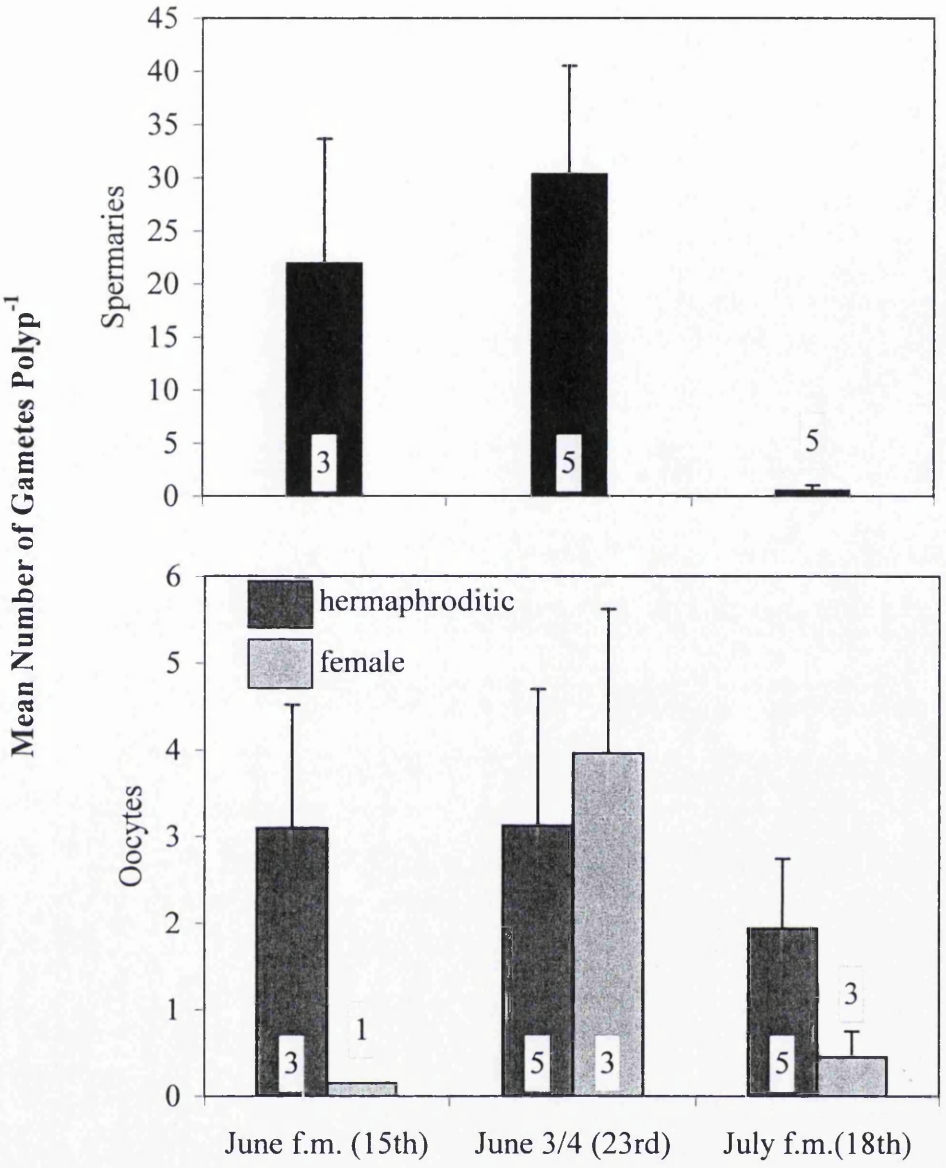


Figure 3.14 : Variation of oocyte and spermary density in *Porites astreoides* colonies from the Outer Lagoon. Bars represent the combined mean number of gametes per polyp from all cores on each sample date (n colonies shown inside the bar, 2 cores taken per colony). Spermary and oocyte density are shown from the same hermaphroditic colonies. There were no male colonies. Error bars represent +1 standard error.

3.5 Discussion

3.5.1 Timing and duration of planula release

Porites astreoides planula release in Bermuda occurs in the summer months of July and August with a small number of planula released in September. The duration of the reproductive season for this species increases with decreasing latitude in the Caribbean, and this is correlated to the narrowing temperature range towards the equator and is discussed in detail in Chapter 5.

3.5.2 Planula release and colony size

The surface area of the *Porites astreoides* colonies of a similar diameter was smallest from the Rim Reef, increased at the Outer Lagoon and was greatest from the Inner Lagoon colonies. This enlargement of surface area from offshore to inshore is caused by the increasing degree of mounds or nodules over the surface of the colony. It is presumed that this is a consequence of the decreased wave action and higher sedimentation along the inshore of the Bermuda platform. Similarly, the growth of *Montastrea annularis* is commonly multi-lobed and "knobby" in areas of increased sedimentation (Barnes, 1973; Rogers, 1990). Less sediment will accumulate on rounded mounds than on concave areas. This advantage outweighs the sediment build up and subsequent reduced fitness of the polyps in the valleys between the mounds. The growth rate of *P. astreoides* is also higher inshore than offshore, partly a further consequence of the favourable conditions of decreased wave energy inshore (Dodge and Vaisnys, 1977; Logan and Tomascik, 1991). Colonies inshore also benefit from the greater food availability along coastal waters (from Beers and Herman, 1969; Morris *et al.*, 1977; Chapter 2), the *P. astreoides* colonies catching a variety of food from fine particulate matter to zooplankton (Lewis and Price, 1975). The colonies with a higher growth rate inshore may have more energy to replicate polyps within colonies as well as at the growing margins.

After reaching the species-defined size at first reproduction, the fecundity per unit area of some coral species initially increases respectively with colony size as more energy is gradually diverted towards reproduction and away from growth (Rinkevich and Loya, 1979a; Kojis and Quinn, 1981b; Babcock, 1984; Babcock, 1988; Van Veghal and Kahmann, 1994b). This relationship then levels off as the 'adolescent' colonies reach adult fecundity levels. The histological study of Chornesky and Peters (1987) did not find any correlation between fecundity per unit area (per polyp) and colony size, but there was a correlation between fecundity and colony age (maximum skeletal thickness). In the present study, every effort was made to normalise the colonies collected by maintaining a similar size and selecting independent colonies that were not part of a fragment, so as not to select immature colonies. Unfortunately, vertical or lateral growth and colony thickness are not related (Chornesky and Peters, 1987) and so estimates of colony age are not possible in the field. However, the surface area of the smallest *P. astreoides* colony in this study was 114 cm^2, and this well exceeded the minimum reproductive sizes previously reported for *P. astreoides* (70 cm^2 McGuire, 1998, in Florida, and 38 cm^2 Chornesky and Peters, 1987, in Jamaica). Thus, all colonies collected in this study were above the known size of first reproduction for *P. astreoides*. Once adult fecundity levels have been reached, overall colony fecundity (gamete production by a whole colony) can increase with colony size, as more polyps become reproductive (for example, *Goniastrea aspera*, Sakai, 1998; and *Pocillopora damicornis*, Richmond, 1987). McGuire (1998) also found that the total number of planulae released by *P. astreoides* colonies statistically increased with colony size, however, the correlation was low. In contrast, in the present study, increasing *P. astreoides* colony size (surface area) was not correlated with the total number of planulae released. It is suggested that the patchy distribution of gametes within *P. astreoides* colonies (Chornesky and Peters, 1987; section 3.4.5), in addition to variable fertilisation success among polyps results in inter-colony variability in fecundity and the overall non-linear relationship between colony size and total planula released.

Planula release occurred from 74% of the *P. astreoides* sampled population, which is a greater percentage than documented for conspecific colonies in Florida (61%; McGuire, 1988). However, planulae production by the monitored colonies was dependent on the month, ranging from 62-93%, which is correlated to inter- and intra-annual variation in

the timing of favourable seawater temperature (Chapter 5). *P. astreoides* colonies are of a mixed sexuality (section 3.5.5), so colonies that did not produce planulae may be sterile, reproductive male colonies, or hermaphroditic colonies that are predominantly male. The lack of inter-zone variation in the number of colonies releasing planulae in this study may indicate a stable sexuality between the colonies, although colony sex was not determined. Previous histological examinations of *P. astreoides* colonies have estimated a lower percentage of the sampled colonies to produce planulae than determined by the actual monitoring of planula release. Soong (1991) found planulae in the tissues of 42-56% of colonies examined in Panama. A comparable percentage of the *P. astreoides* population sampled in Jamaica was reproductive with planulae found in 50% of female colonies and 46% of male colonies (Chornesky and Peters, 1987). Estimates from tissue dissections of colony fragments may be a mis-representation of fecundity as gamete density has been shown to be patchy within coral colonies for *P. astreoides* (Chornesky and Peters, 1987) and also in other colonies (Rinkevich and Loya, 1979a; Wallace, 1985; Van Veghal and Kahmann, 1994b). The percentage of *P. astreoides* colonies releasing planulae in this study is comparable to *Agaricia humilis* in the Caribbean of which 78% of the colonies planulated (Van Moorsel, 1983). In contrast, only 26% of the colonies of the sibling species *Agaricia agaricites* released planulae (Van Moorsel, 1983). Thus, the proportion of a population that successfully completes gametogenesis to planula production can be variable between similar species occupying the same habitat. Planula production can also be highly variable within a species, for example, the percentage of *Pocillopora damicornis* colonies from a population at Heron Island, Great Barrier Reef that released larvae ranged from 10-80% over the peak reproductive months (Tanner, 1996).

The actual number of planulae released per reproductive colony can be highly variable, so estimates of the percentage of colonies that planulate may not accurately represent reproductive effort. The calculation of the number of planulae produced per polyp leads to estimates of the percentage of polyps producing planulae within a colony. It was calculated in this study that 2-21% of the polyps of reproductive *P. astreoides* colonies released planulae. This is a higher fecundity than McGuire's (1998) observation that only 1-10% of the polyps of colonies in Florida released planulae. However, her estimates are likely an under-estimation as she calculated polyp density on fixed tissue that would have shrunk after the histological processing (section 3.4.2). Other data

available for *P. astreoides* are from Chornesky and Peters (1987) who recorded between 0.03-0.62 planulae polyp^{-1} from histological sections, which equates to 3-62% of polyps producing planulae. As already mentioned, histological examination may mis-represent planula production by only looking at colony fragments, thereby assuming a uniform distribution in fecundity. Histological results may also over-estimate planula production per month as some planulae may not be released over a planulating period, such has been reported for the Caribbean species *Favia fragum* (Szmant-Froelich *et al.*, 1985a). Retained planulae are presumably either released on the next period of planulation or resorbed. Estimates of the number of oocytes per polyp in this study (section 3.4.5) ranged from a mean of 2.68 oocytes polyp^{-1} in female colonies (max 9.05 oocytes polyp^{-1}) and 2.89 oocytes polyp^{-1} in hermaphroditic colonies (max 8.15 oocytes polyp^{-1}). Oocytes of all developmental sizes were recorded in this fecundity estimate and so only a proportion of those will be mature for fertilisation each month. However, the low percentage of polyps producing planulae does imply that many oocytes are not fertilised and would presumably be resorbed at the end of the reproductive season to retain nutrients.

Planulae per polyp estimates used in this study assume that only one *P. astreoides* planula is released by one polyp. This is credible on account of the relatively large diameter of ~0.5 mm of mature planulae compared to the limited space available within the small polyp size of *P. astreoides* (corallite diameter of 1.2-1.6 mm, Veron, 2000). For some species there is a relationship between the number and/or size of planulae produced and the polyp size. The polyps of *Favia fragum* at ~5 mm in diameter are larger than those of *P. astreoides* and planula width is correspondingly larger at >0.5 to 1 mm (Szmant-Froelich *et al.*, 1985). A proportion of *F. fragum* polyps produced more than one planula and these were smaller at ~0.25 mm width. The planula of *Pocillopora damicornis* have a mean diameter of 0.88μm and 99% of the polyps brooded just one planula (Tanner, 1996). The slightly smaller planulae of *Seriatopora hystrix* (mean 0.63μm) were brooded in pairs in the polyps, whilst the mean planula diameter of *Stylophora pistillata* is only 0.56μm and up to eight were brooded per polyp (Tanner, 1996).

3.5.3 Inter-zone variability to lunar periodicity

The planulae of many brooding species are synchronously released over a period of days in the spawning month, on the cue of the lunar cycle (Harriott, 1983; Richmond and Jokiel, 1984; Stoddart, 1985; Jokiel, 1985; Szmant-Froelich *et al.*, 1985; Kojis, 1986a; Johnson, 1992; Tanner, 1996). In this study, a peak in the planulation of *Porites astreoides* colonies from the Rim Reef zone occurred around the new moon. McGuire (1998) also monitored *P. astreoides* planula release and similarly found an increase in the reproductive activity of colonies over the new moon period. Chornesky and Peters (1987) recorded planula abundance in the coral polyps by histological examination, also noting a peak prior to the new moon. Soong (1991) correspondingly recorded a decrease in the number of planulae present in the coral polyps over the new moon period and between the new moon and the first quarter moon phase.

The synchrony of planulation of *P. astreoides* in this study, however, varied among the colonies, dependent on the reef zones. Whereas the reproductive activity of colonies collected from the Rim Reef peaked around the new moon, colonies from the Outer Lagoon released planulae for an extended period before the new moon and some colonies were active at the start of the monitored period, ten days before the new moon. In contrast to the Outer Lagoon and Rim Reef, the Inner Lagoon colonies showed a protracted period of planulation and both the percentages of colonies releasing planulae and the total number of planulae released decreased around the new moon. It is proposed that this inter-zone variation in lunar periodicity be caused by the different environmental conditions at the reef zones altering the external cues controlling synchronisation.

Sedimentation rates at the Inner Lagoon reefs of Bermuda are over three times greater compared to the other reef zones, and there is a gradient of decreasing turbidity moving across the lagoon toward the Rim Reef zone (Bodungen *et al.*, 1982; CARICOMP, 1997; Chapter 2). The increased turbidity inshore is partly caused by higher levels of productivity and land run-off (Beers and Herman, 1969) and also by re-suspension from the benthic sediment in the shallow water (Bodungen *et al.*, 1982). Furthermore, the Inner Lagoon reefs are located next to a shipping channel and the passing of large ships leaves plumes of disturbed sediment in their wake that can be moved to the Inner

Lagoon reefs by currents (S.R. Smith, unpub. data; A. Wartham, unpub. data). Increased turbidity in the water column will reduce light penetration levels. Night irradiance (moonlight) was experimentally shown to be the specific lunar cue for the synchronisation of planula release in *Pocillopora damicornis* (Jokiel *et al.*, 1985). Under local conditions, *P. damicornis* colonies planulate over alternate phases of the lunar cycle, and this is thought to be caused by variability in night irradiance. At Lizard Island on the Great Barrier Reef, planulation occurs in the winter months timed to the full moon period of strong night irradiance (Harriott, 1983). Planulation continues in the summer months, although there is a decrease in lunar periodicity with extended periods of planula release. Increased cloud cover and rain over the Australian summer months obscures the bright phases of the moon, minimising the amount of moonlight. The weakening of the cue of night irradiance to the *P. damicornis* colonies in the summer is suggested to prolong the periods of planulation (Jokiel *et al.*, 1985). On the inshore reefs of the Bermuda platform, the reduced light attenuation caused by the increased turbidity there will similarly obscure night irradiance levels. If night irradiance is an external factor acting on the timing of planula release of *Porites astreoides*, as it is for *Pocillopora damicornis*, then the reduction in the strength of this cue will cause the observed breakdown in synchrony of planulation from the Inner Lagoon colonies. The decreasing gradient of turbidity levels with distance moved offshore to the Outer Lagoon and Rim Reef corresponds with the observed tightening in the synchrony of planula release at these offshore reef zones.

Pocillopora damicornis also releases planulae over different moon phases in Hawaii, although these phase shifts occur simultaneously within the same population (Harriott, 1983; Richmond and Jokiel, 1984; Jokiel, 1985). Richmond and Jokiel (1984), postulated that the variation of lunar periodicity among the population may in part be related to living in an unstable reef flat environment of variable abiotic factors. The Inner Lagoon zone of the Bermuda platform experiences the greatest temperature range of the reef zones (15.5-31°C), reaching the extremes of coral growth and survival in Bermuda (Chapter 2). Combined with the increased sedimentation, the *P. astreoides* colonies from the Inner Lagoon endure greater environmental stress then their conspecifics offshore. Stress to the colonies may also vary at small spatial scales on the reef affecting the energy availability towards reproduction and planula growth. If

planulae are not released until they are mature, a variation in developmental times will cause a break down in synchrony. There is also the possibility that planula release was disturbed in the aquarium from the stress to the colonies and, even though every effort was made to reduce external lights, there was inevitably some alteration of the natural light regime in aquaria. Colonies were not monitored *in situ* in this study, although *P. astreoides* colonies monitored in the lab and in the field in Florida planulated over similar time periods (McGuire, 1998).

A study by Brazeau *et al.* (1998), using DNA markers, showed that a relatively high percentage of the planulae of *Porites astreoides* and *Favia fragum* are produced by self-fertilisation (49% and 34% respectively). *Porites astreoides* colonies are hermaphroditic, female or male and, among hermaphroditic colonies, polyps can also be male, female or hermaphroditic (section 3.4.5 and 3.5.5). Rates of self-fertilisation in *P. astreoides* and *F. fragum* were variable and the origin of planulae from colonies ranged from being produced completely by out-crossing to almost entirely the product of self-fertilisation (Brazeau *et al.*, 1998). This would be implemented by the sex of the colony with planulae from female colonies or hermaphroditic colonies that are primarily female relying on cross-fertilisation of oocytes, whereas true hermaphroditic colonies may engage predominantly in selfing. It is possible, although has not been studied, that synchrony of gametogenesis may vary dependent on the level of self or cross-fertilisation. Female and male colonies relying on cross-fertilisation may exhibit strong synchronisation to gametogenesis to ensure maximum fertilisation. The development of gametes of hermaphroditic colonies would benefit from intra-colony synchronisation but may not be as highly constrained to the co-ordination of gametogenesis between colonies. If this assumption were correct, this leads to an interesting speculation of colony sex variation and the degree of self-fertilisation occurring between the sites possibly influencing the degree of synchronisation of planula release. However, the sex of the *P. astreoides* colonies monitored for planula release in this study was not determined and patterns in gametogenesis and the timing of fertilisation could not be defined from the small sample sizes (section 3.5.5).

It is possible from the results that *P. astreoides* planula release from the Inner Lagoon colonies occurred outside the monitored periods. An initial study was performed to examine whether planulation of *P. astreoides* in Bermuda occurred over the full moon

period. There were no planulae released for four days either side of the July or August 1998 full moons, although colonies were only monitored from the Outer Lagoon and the Rim Reef. A further study is needed observing *P. astreoides* colonies from the Inner Lagoon to confirm whether the timing of planula release for this population is continuous throughout the lunar cycle or whether planulation decreases or stops over the full moon. An interesting result was the substantial planula release from two colonies from the Inner Lagoon at the end of the July 2000 new moon period on the first-quarter moon phase. Monitoring of all colonies continued in aquaria and planula release was extended from these colonies for a further eight days until the August full moon period. These colonies had released a small number of planulae prior to the July new moon, although the reproductive effort was greater at the beginning of August. Several *P. astreoides* colonies (collected from the Rim Reef) held in aquaria in a previous study in Bermuda planulated consecutively over two months (de Putron, unpublished BSc Honour thesis). The timing of the release of planulae was similar to the present study, occurring over the new moon in the first month and then earlier, over the full moon period in the second month. The disruption of synchrony over the second planulating period is likely a stress response of being held in aquaria, related to the unnatural conditions and possible confusion of night time irradiance from external light pollution. The *P. astreoides* colonies monitored in Florida did not planulate over a second month (McGuire, 1998). Colonies of *Favia fragum* in Puerto Rico also released planulae over successive months (Szmant-Froelich *et al.*, 1985a), as did *Manicina areolata* in Panama, although reproductive effort was lower from the colonies over the second month of study (Johnson, 1992). The release of planulae over successive periods from isolated colonies held in aquaria may be from planula retention (Szmant-Froelich *et al.*, 1985), or the result of self-fertilisation (Brazeau *et al.*, 1998). The reliance on selfing and the lack of cross-fertilisation may also be the cause of a reduction in the number of planulae released over successive planulating periods and a disruption in synchrony.

The release of planulae from the majority of *P. astreoides* colonies was over several days, even from colonies collected from the Rim Reef where synchronisation was tighter than the other reef zones. Protracted periods of planulation over the lunar cycle similarly occurs in other brooding species (Van Moorsel, 1983, for *Agaricia* spp.; Kojis, 1986a, for *Acropora* spp.; Tanner, 1996, for *Pocillopora damicornis* and *Seriatopora hystrix*). Other species show no relationship between planula release and the lunar

cycle, for example, *Acropora* spp. from Heron Island on the Great Barrier Reef, in which planulation was synchronous from some colonies but predominantly continuous and not related to abiotic factors (Kojis, 1986a). Other species with a brooding mode of reproduction do not show lunar periodicity of planula release, for example *Porites porites* (Tomascik and Sander, 1987) and *Stylophora pistillata* (Rinkevich and Loya, 1979b). If planula development times are synchronised within and among colonies, lunar periodicity to planulation will be a reflection of the synchrony of gametogenesis. *P. astreoides* colonies do not show synchronous oocyte development, and lunar periodicity to spermatogenesis occurred for only some of the studied populations (Szmant, 1986; Chornesky and Peters, 1987; Soong, 1991; this study, section 3.4.5). Oogenesis and spermatogenesis both showed lunar periodicity in *Favia fragum* colonies sampled in Puerto Rico, resulting in synchronised ovulation and shedding of sperm (Szmant-Froelich *et al.*, 1985). Thus, planula release from *F. fragum* colonies exhibited a tighter synchrony than shown by *P. astreoides*, occurring 6-15 days after the new moon. The release of planulae from *Manicina areolata* is further synchronised, occurring mainly within just two days of the new moon, and gametogenesis is correspondingly under a strict lunar control (Johnson, 1992).

Synchrony of planulation in brooding corals may reduce mortality of propagules by predator satiation (Johnson, 1992). Lunar periodicity to planula release will also influence retention or dispersal to the local area in association with the tidal amplitude of different lunar phases. The planulation of brooding species that are synchronised to the lunar cycle are mainly timed either to the full moon or new moon periods, and this may be selected for as the high tidal amplitude over the spring tides would facilitate dispersal (Johnson, 1992). In contrast, many broadcasting species synchronise the release of gametes to the last quarter moon phase, which is the time of tidal slackness to promote gamete mixing rather than dissipation (Babcock *et al.*, 1986).

3.5.4 Mucous sheet formation

The formation of mucous sheets on *Porites astreoides* colonies did not follow any clear patterns over time or among the reef zones over the 12 day monitored period (six days either side of the new moon). Coffroth (1991) monitored colonies *in situ* and for a longer period of three months and showed mucus to occur over a cyclical pattern for *P. astreoides* and *P. furcata* colonies in Panama. The formation of mucous sheets was periodic over the lunar cycle with an increase in the percentage of *P. astreoides* colonies with mucous sheets over the first quarter moon phase and an increase around the full moon for *P. furcata*. The lack of a pattern to mucous sheet formation in this study may be a factor of the short period of monitoring as colonies were only scored for mucous sheets until a day before the first quarter moon phase. Induced stress to the colonies from holding them in the aquaria may also be a factor disrupting any cyclical pattern. Corals depend on water movement for cleaning and feeding and the disruption of natural flow around colonies may promote mucous sheet formation to aid these processes (Bak and Elgershuizen, 1976; Coffroth, 1985). Water movement in the 'planulae collectors' was encouraged by the inflow at the base of each Tupperware tub but this would be lower and less erratic than the water flow levels on the reef. Mucous sheet formation in some poritid corals has been shown to be influenced by increased levels of sedimentation, reduced salinity (Bak and Elgershuizen, 1976; Coffroth, 1985; Kato, 1987), and increased temperature (Kato, 1987; Coffroth, 1991). Sedimentation and salinity were stable within the aquaria holding the *P. astreoides* colonies. The temperature of the aquarium seawater remained intermediate between the reef zones and followed the *in situ* temperature profiles (Chapter 5). The daily range in temperature of the aquarium seawater was greater than that of the reef water, as the smaller water mass rapidly warmed and cooled with the fluctuating air temperature (Chapter 5). However, there was no consistent trend of increased presence of mucous sheets over the time held in aquaria that would indicate increasing levels of stress to the colonies.

The significance of the possible lunar cycle to mucous sheet formation is through the effect on planulae and/or sperm release and fertilisation. The presence of mucous sheets in *P. furcata* around the full moon is at the same time as sperm release, which is also under a lunar control for this species (Coffroth, 1991). The presence of a mucous sheet on the colony would presumably inhibit the release and transfer of sperm, thereby

reducing cross-fertilisation rates. Planula release of *P. astreoides* colonies in Panama has been timed to the new moon, and planulae were not found in the tissues over the first quarter moon phase (Soong, 1991). The timing of mucous sheet formation on *P. astreoides* colonies in Panama over the first quarter moon phase (Coffroth, 1991), would therefore not affect planula release. There was no lunar control to gametogenesis discernible for *P. astreoides* colonies studied from Panama (Soong 1991) or in this study (section 3.5.5), although mature sperm were most abundant around the time of full moon from colonies in Puerto Rico (Chornesky and Peters, 1987). Any correlation between planula release, mucous sheet formation and the lunar cycle in this study is difficult to interpret from the limited period over which colonies were monitored. However, the formation of mucous sheets was not consistently related to the number of planula released.

3.5.5 Colony sexuality and patterns of gametogenesis

The eight *Porites astreoides* colonies sampled for histological examinations of reproductive structures were either female or hermaphroditic. Chornesky and Peters (1987) also recorded an absence of male colonies in the *P. astreoides* population studied in Puerto Rico and they adopted the botanical term "gynodioecy" for the population, meaning the presence of only female and hermaphroditic colonies (Dunn, 1974). Szmant (1986) and Soong (1991) found a mixed sexuality of male, as well as female and hermaphroditic colonies, in the *P. astreoides* populations studied in Jamaica and Panama respectively. The majority of the hermaphroditic colonies in these populations were either predominately male or female. This led to the speculation that all colonies were hermaphroditic and the apparent absence of gametes of one sex from a colony was an error of sampling colonies with a patchy distribution of each gamete type (Szmant, 1986). A strongly skewed sex ratio also occurred in three of the five hermaphroditic colonies in this study and oocyte density from the female colonies was highly variable from the cores, thereby also indicating unevenness of gamete density within a colony. Mixed sexuality within a coral population may also be caused by the species exhibiting sequential hermaphroditism, younger colonies either maturing first as male (protandrous) or as female (protogynous) before later changing sex or developing both sexes. Protandrous hermaphroditism has been reported for several coral species and is

presumed to reflect the smaller amount of energy needed for the production of spermaries over oocytes permitting more energy for growth of the juvenile colony (Rinkevich and Loya, 1979a; Kojis and Quinn, 1981a; Harriott, 1983a; Kojis and Quinn, 1985). However, the mixed sexuality of the *P. astreoides* colonies sampled by Chornesky and Peters (1987) was found to be temporally stable and gender was not a function of age. A mixed sexuality has also been reported for the brooding species *Agaricia agaricites* and *A. humilis* (Delvoye, 1988), although this may be a consequence of taxonomic differences with a suspected presence of gonochoric sub-species (Harrison and Wallace, 1990).

Populations of *Galaxea fascicularis* in the Pacific also have both female and hermaphroditic colonies, although the eggs of the hermaphroditic colonies are non-viable and solely serve as a flotation device to bring the sperm to the surface during spawning in this broadcasting species (Harrison, 1988). This "pseudo-gynodioecy" breeding system will facilitate cross-fertilisation and decrease the dilution of gametes in a functionally gonochoric population. It is not known whether this strategy developed from true hermaphroditism in the species, although it is proposed that hermaphroditism is the ancestral condition in scleractinian corals (Szmant, 1986). Gonochorism is believed a selective trait to ensure outcrossing (Szmant, 1986). Reproductive success, however, is compromised in some gonochoric species as the inadequacy of sperm availability and incidences of sperm dilution constrains successful fertilisation (Denny and Shibata, 1989; Brazeau and Lasker, 1992; Lasker and Stewart, 1992; Levitan and Petersen, 1995; Lasker *et al.*, 1996a). The occurrence of a mixed sexuality in a population may be advantageous to ensure additional fertilisation through self-compatibility in hermaphroditic colonies to the necessary occurrence of cross-fertilisation from gonochoric colonies, thus promoting some degree of outcrossing. Self-fertilisation may also be important when population densities are low (Charnov, 1982; Szmant, 1986) or when the colonies are under stressful conditions (Tomascik and Sander, 1987). A low incidence of hermaphroditism occurred in the predominantly gonochoric species *Porites cylindrica* (as *P. andrewsi*, Kojis and Quinn, 1981a), *Astrangia danae* (Szmant-Froelich *et al.*, 1980), and *Porites porites* (Tomascik and Sander, 1987), the latter believed to be a response to pollution stress.

Developmental patterns in gametogenesis could not be determined from the small histological sample sizes in this study. Oocytes of all sizes were present throughout the samples collected over the lunar cycle, which is a similar result to previous studies on the gametogenic cycle of *P. astreoides* (Chornesky and Peters, 1987; Soong, 1991). In contrast, spermatogenesis in *P. astreoides* colonies from Jamaica was synchronous with spawning, occurring just prior to the full moon (Chornesky and Peters, 1987), although Soong (1991) found spermaries throughout the lunar cycle in the population studied in Panama. Frequent sampling over the reproductive months would be needed to determine whether gametogenesis is under lunar control in Bermuda. The maximum oocyte diameter recorded in *P. astreoides* colonies was 135 μm, with a mean fecundity of 2.9 oocytes polyp^{-1}. This is a result comparable to Chornesky and Peters (1987), who recorded maximum oocyte diameter from colonies in Jamaica to be 130-170 μm, and the greatest median fecundity over the sampled months to be 2.3 oocytes polyp^{-1}. In Panama, maximum recorded oocyte diameter was larger, at 200 μm, and fecundity estimates of the large eggs was greater than in Bermuda, being a mean of 10 oocytes polyp^{-1} (Soong, 1991). Maximum oocyte diameter of *P. astreoides* sampled in Puerto Rico was only 50 μm and there was a high abundance of the small oocytes with a mean of 20 oocytes polyp^{-1} (Szmant, 1986). Thus, fecundity of *P. astreoides* colonies is variable among the Caribbean populations studied and between Bermuda and the Caribbean. Only two planulae were found in the Bermuda colonies on the last sample date just prior to the July full moon. There was no evidence of mesentery formation in these planulae, although they were of a medium size, being 250-300 μm (after shrinkage of 20-30% from the histological process). Released planula diameter ranged from ~1.5 mm when swimming to 1 mm when stationary and round. Thus, it is likely that these planulae were maturing for release after the August new moon, two weeks later.

3.6 Summary

Porites astreoides planula release in Bermuda occurs over the summer months of July and August with smaller numbers released in September. The colonies selected for the study were all above the size of first reproduction previously recorded for the species and total number of planula released was not correlated with colony size. The extent that planula release of *P. astreoides* was synchronised to the lunar cycle varied according to the reef zone in Bermuda. An increase in planulation occurred over the new moon from the offshore colonies at the Rim Reef zone. There was a gradient of declining synchrony of planula release with distance inshore to the Inner Lagoon zone. It is proposed that this is a consequence of increasing turbidity inshore that disrupts the synchrony cue of night time irradiance. Superimposed on the overall degree of synchronisation to lunar periodicity at the reef zones was a variation among the colonies in the timing, duration and intensity of planula release. It is speculated that this could be a consequence of colony sex and the reliance on self- versus cross-fertilisation. The *P. astreoides* populations studied in the Caribbean and in Bermuda persistently show the presence of a mixed sexuality of female, hermaphroditic and sometimes male (in the Caribbean) colonies. The necessity of single sex colonies to outcross along with the proven ability of this species to engage in selfing suggests that both methods of fertilisation are important. The degree of selfing versus outcrossing could potentially affect the success and timing of fertilisation causing a variation in reproductive effort and planula release times. The *P. astreoides* colonies held in aquaria formed mucous sheets over the monitored period although there was no detectable cyclical pattern to this formation, and mucous sheet presence was not consistently correlated with the timing or intensity of planula release.

Chapter 4: A study of the reproduction and population ecology of *Pseudoplexaura porosa* (Gorgonacea: Plexauridae) in Bermuda.

4.1 Introduction

Gorgonian species are common components of many coral reef communities in the Caribbean. Despite their importance, literature on their reproductive biology is sparse (for review see Chapter 1) as the surge in research on coral species over the past 20 years has largely been concerned with scleractinian corals (for reviews see Szmant-Froelich *et al.*, 1983; Fadlallah, 1983a; Harrison and Wallace, 1990; Richmond and Hunter, 1990; Richmond, 1997; Chapter 1). This study examines the reproduction and population ecology of the gorgonian plexaurid *Pseudoplexaura porosa* in Bermuda.

There has been no previous documentation of gorgonian reproduction from Bermuda. Research on coral biology in Bermuda is of particular interest because of the northerly location of this pseudoatoll in the Atlantic (32°N). The coral populations in Bermuda survive an extreme annual seawater temperature range for coral species of 15-31°C. In addition, within the 18 km wide Bermuda platform there are different temperature profiles and also gradients of wave energy and sedimentation. These environmental parameters separate the North Lagoon into three broad physiographic reef zones: the Inner Lagoon, Outer Lagoon and Rim Reef (Chapter 2). As well as documenting the reproductive cycle of *P. porosa* in the sub-tropical environment of Bermuda, this study examines variation in colony abundance, sex and morphology at the three reef zones in relation to the different physical conditions. Variation in the reproductive effort of conspecifics occurring within reef zones is later examined in relation to the different temperature profiles (Chapter 5).

The distribution and abundance of shallow-water gorgonian species has been related to several environmental factors, these primarily being substratum preferences (Bayer, 1961; Kinzie, 1973; Lasker and Coffroth, 1983), depth and wave energy (Bayer, 1961; Kinzie, 1973; Birkeland, 1974; Yoshioka and Yoshioka, 1989), competition (Wahle, 1980), and predation (Lasker *et al.*, 1983; Lasker, 1985). Population structure is also defined by life history characteristics, such as the reliance on vegetative reproduction (Lasker, 1983; Lasker, 1988; Lasker, 1990), and determinants of successful levels of settlement and recruitment (Goldberg, 1973; Lasker and Coffroth, 1983; Gotelli, 1988; Lasker, 1991). *Pseudoplexaura porosa* (Houttuyn, 1772) of the order Gorgonacea, the sub-order Holaxonia and the family Plexauridae, occurs commonly throughout the Caribbean (Sterrer, 1986; Humann, 1993). In Bermuda, *P. porosa* is abundant on both inshore and offshore reefs (Sterrer, 1986; Cavaliere *et al.*, 1987) and dominated the gorgonian fauna of 16 out of 22 surveyed reef sites (Smith *et al.*, 1984). Abundance of *P. porosa* in terms of biomass has not been documented, although it should be noted that colonies can obtain a large size (up to 250 cm high in Panama, Kapela and Lasker, 1999; and 225 cm in Bermuda, Sterrer, 1986,) and visually dominate many reefs (Lasker and Coffroth, 1983; pers. obsv.).

There are two other common described species of the genus *Pseudoplexaura* in Bermuda and the Caribbean: *P. flagellosa* (Houttuyn, 1772) and *P. wagenaari* (Stiansy, 1941). A fourth species, *P. crucis* was distinguished in the Virgin Isles, and may occur throughout the Antilles (Bayer, 1961). A morphologically distinct *Pseudoplexaura* species has been observed from Panama (Lasker *et al.*, 1996). This potential fifth species most closely resembles *P. porosa*, and confirmation of separate species status is awaiting genetic analysis (H.R. Lasker, pers. comm.). All members of this sea rod genus are characterised by having an infrequent dichotomous branching pattern and prominent polyps that can retract fully to leave round-oval pores without raised calyces (Sterrer, 1986; Humann, 1993). Colony colour is variable, ranging from light brown to reddish purple when the polyps are extended, which is generally throughout the day and nights (Wainwright, 1967), and light purple to grey when the polyps are retracted. Separation to species by external morphology is by pore diameter and the spacing of the pores along the branches (Bayer, 1961; Sterrer, 1986). Colonies of *P. porosa* have the largest pore diameter of 1-1.5 mm and the pores are separated by less than their own diameter. *P. flagellosa* and *P. wagenaari* have a smaller pore diameter of 0.5-1 mm

which are spaced further apart, separated by more than their own diameter. Colony height has also been used as a diagnostic feature with *P. porosa* colonies being taller, reaching 2.25 m in Bermuda. Colonies of *P. flagellosa* are moderate sized and generally <1 m, and *P. wagenaari* colonies are smaller, being <30 cm.

Further species clarification of gorgonians, which lack the external skeletal morphology of scleractinian corals, is by the appearance of the calcium carbonate spicules (sclerites) embedded in the tissues. The primary description of the spicule morphology of *Pseudoplexaura porosa* is in Bayer (1961). Recent investigations have compared and contrasted Bayer's account of the characteristic spicules of *P. porosa* with spicule analysis of *P. porosa* colonies sampled from Bermuda (A. Neill, J. Gelerter and J. Bilewitch; unpublished data carried out over 1998-2000). These studies confirm the presence of five main spicule types in Bermuda *P. porosa* colonies, as originally described by Bayer (1961). Arguments have arisen as to a potential sub-species also occurring in Bermuda from the differences in the proportions of these spicules in the colonies compared to the published data. Therefore, all colonies monitored for this reproduction study were identified to be of the same species using spicule analysis. A summary of the results of past unpublished data is given in addition to the present results to provide an overview of the spicule morphology of *P. porosa* in Bermuda.

The reproductive cycles of all Caribbean broadcasting gorgonian species studied to date are seasonal occurring over the summer months of warmer seawater temperatures (Goldberg and Hamilton, 1974; Brazeau and Lasker, 1989; Brazeau and Lasker, 1990; Beiring and Lasker, 2000). The reproductive cycle of *Pseudoplexaura porosa* has been documented in Panama (Lasker *et al.*, 1996; Coma and Lasker, 1997a; Kapela and Lasker, 1999). The colonies are gonochoric with an even sex ratio and are broadcast spawners. In the studied Panama population, spawning occurs 5-6 days after the full moons of June until August with a weak spawning in September. Spawning continues for 2-4 days with individual colonies partaking in 1-2 nights of intense spawning and 1-2 nights of a weakened effort. A morphologically distinct *Pseudoplexaura* species in the Panama population spawned on the same nights as *P. porosa* (Lasker *et al.*, 1996). This study investigates whether the reproductive season, spawning and sex ratio of the *P. porosa* population surviving in the sub-tropical environment of Bermuda is similar to the documented research in Panama. Variation in fecundity is also examined in the

Bermuda population, both within colonies and also between colonies; the latter examined as a function of polyp size.

4.2 Objectives

This study addresses the following questions on the reproduction and population ecology of *Pseudoplexaura porosa* in Bermuda:

1. What are the characteristic spicules for *P. porosa* in Bermuda?
2. What is the abundance of *P. porosa* colonies across the three physiographic reef zones of the Bermuda platform?
3. Are there differences in sex ratio and colony height between the *P. porosa* colonies at the different sites across the reef zones?
4. What is the reproductive cycle of *P. porosa* in Bermuda?
5. Does the polyp volume of *P. porosa* colonies vary between the different sites across the reef zones and is there a relationship between fecundity and polyp volume?
6. Does fecundity vary within *P. porosa* colonies?

4.3 Methods

4.3.1 Spicule analysis of tagged colonies

Over the study period of 1998-2000 between five and eight *Pseudoplexaura porosa* colonies were tagged at replicate reef sites representing the three reef zones of the Bermuda North Lagoon: the Inner Lagoon, Outer Lagoon and Rim Reef (Figure 2.1, Chapter 2). The colonies were repetitively sampled to monitor gamete development (section 4.3.3) and the duration and timing of spawning (section 4.3.4). The *P. porosa* colonies that were tagged were selected as mature (>50 cm tall; Kapela and Lasker,

1999) and being of approximately the same height (80-100 cm). Species identification in the field was by the characteristic diameter and wide spacing of the pores (section 4.1). Confirmation of species similarity was made by the presence of diagnostic spicules within the tissues. Two branch samples, approximately 8 cm long were collected from each tagged *P. porosa* colony at all the sites. A thin cross-section (2-3 mm) was cut at 5 cm from each branch tip. The tissue was dissolved in concentrated bleach (1-2 days) and the remaining spicules from each section were washed in distilled water, dehydrated in ethanol, mounted on slides and dried. The slides were examined under a compound microscope for the presence of five characteristic spicules described by Bayer (1961) for *P. porosa* and the absence of any diagnostic spicules for the sibling species *P. flagellosa* and *P. wagenarri*. Spicule presence from each slide preparation was classified using an abundance scale. The characteristic spicules used for *P. porosa* identification are described below and illustrated in Figure 4.2 and 4.3.

1. Colourless spindles, which have a fusiform shape with prominent but widely spaced tubercles.
2. The unilaterally spiny spindle, which is the same size as the colourless spindle with strong spines along one side.
3. Clubs, which are shorter spicules that are wider at one end. Spines occur all over and are particularly developed at the wider end of the club.
4. Purple spindles, which are similar in shape to the colourless spindles, again with widely spaced tubercles.
5. Occasional spindles forming 3- or 4-rayed bodies, usually purple and less frequently seen as colourless.

The differentiating spicule characteristics for *P. porosa* are the wide spacing tubercles of the colourless and purple spindles and the presence of the unilaterally spiny spindle and the spiny clubs. The congeneric species of *P. flagellosa* and *P. wagenarri* have closely spaced tubercles on the spindles, do not possess the unilaterally spiny spindle, and their club ends are more rounded and foliate in appearance (see p.108 in Bayer, 1961). The branching 3-4 rayed spindles are commonly purple in *P. porosa* species and are more often colourless in the congeneric species, although are not a diagnostic feature. A sixth spicule type are the abundant purple capstans, which are smaller spicules with two uniform whorls of tubercles at each end. The purple capstans are present in all *Pseudoplexaura* species.

4.3.2 Population Study

A population survey was performed to determine the abundance, size and sex ratio of *Pseudoplexaura porosa* colonies at the replicate study sites of the three reef zones across the Bermuda North Lagoon: the Inner Lagoon, Outer Lagoon and Rim Reef (Figure 2.1, Chapter 2). The characteristics of the Bermuda platform and the physiographic reef zones are described in Chapter 2. The heights of all colonies >50 cm tall were measured within a total area of 100 sq m at each of the six study sites by surveying 1 m either side of five randomly deployed 10 m long transects. A branch sample of 10 cm length was taken from the measured colonies and examined under a dissecting microscope to establish the colony sex by the occurrence of either eggs or spermaries. The surveys were performed during July and August 1999 when sex determination was possible from the presence of mature gametes.

4.3.3 Gamete development

Gamete development of *Pseudoplexaura porosa* was monitored by the repetitive sampling of tagged colonies from an Outer Lagoon and Rim Reef site over April to November 1998 (see Appendix 4.1A for sampling schedule). In April 1998, eight colonies were randomly sampled at Crescent C of the Outer Lagoon and eight colonies from Hog Breaker at the Rim Reef (Figure 2.1, Chapter 2). Mature oocytes were not present. Repetitive Sampling then began at the beginning of May 1998 from five tagged colonies at each site and was continued approximately every two weeks when possible until September, and then less frequently thereafter until the end of November 1998. All sampled colonies were of the same height (80-100 cm) and were confirmed to be *P. porosa* from spicule analysis (section 4.3.1).

Sampling was carried out by SCUBA divers using pliers to collect a branch tip approximately 15 cm long from each colony. The samples were fixed in 9% seawater formalin for 36hr and then transferred to 70% EtOH before examination. Gamete development was monitored by cross sections of approximately 5 cm thickness along the branches made by a sharp razor blade. These cross sections result in longitudinal

cuts through the polyps surrounding the axial rod. Whole polyps were dissected out and developing gametes observed using a dissecting microscope at x25 and x50 magnification.

In addition to examination by hand sections, gamete development of representative samples from the tagged *Pseudoplexaura porosa* colonies was further described by histological analysis. A branch sample was collected from a randomly selected tagged male and female colony at Crescent C and Hog Breaker on several sample dates between May and August 1998. The branch samples were fixed in 9% seawater formalin (36hr) and then a 1 cm cross section was cut from each sample by a sharp razor blade at a distance of 8-10 cm from the branch tips. These sections were decalcified in a solution of 10% formic acid that was changed every 12 hours. Decalcification was complete within approximately 36hr when the samples were washed thoroughly and then dehydrated in a gradation series of ethanol dilutions, cleared and then embedded in paraplast. Serial cross sections were made at 8μm thickness through the branch resulting in longitudinal sections along the polyps. Sections were mounted on slides and stained with Mallory's Triple Stain (Humason, 1962; Grimstone and Skaer, 1972) and observed under a compound microscope for descriptive accounts.

4.3.4 Spawning

Months of spawning

The spawning months of the tagged colonies was inferred from the disappearance of mature gametes in samples collected on successive dates around the ostensible spawning times. The study in 1998 revealed that *P. porosa* colonies in Bermuda spawned on a similar lunar cycle as in the Caribbean, between the full moon and third quarter moon phase (Lasker *et al.*, 1996a; Kapela and Lasker, 1999), and that mature gametes were present over the summer months of July/August until September/October. Over 1999 and 2000, the sampling regime was increased to include replicate study sites representing the three reef zones: the Inner Lagoon, Outer Lagoon and Rim Reef (Figure 2.1, Chapter 2) to determine the months of spawning and examine for inter-

colony and intra-zone variation in spawning. The sample size at each site included those colonies monitored in 1998 with the number of additional colonies varying between five and eight per site (Appendix 4.1C). Samples were collected as described in section 4.3.3 as close as feasible to the full moons of May until October each year (Appendix 4.1B). The presence of mature gametes in the colonies represented the potential for spawning to occur that month around the third quarter moon. In all months of 1999 and 2000 when there was only one sample date just prior to the full moon, two branches were collected from each colony. If the branch was immature, the other branch was checked for gamete presence to allow for intra-colony variability in maturation. Only one extra branch was collected to limit stress to the colonies.

Timing of spawning

Samples were collected from ten additionally tagged colonies from the Inner Lagoon (five from Tynes West and five from Tynes East) around the July and August 1999 full moon to determine the exact day of spawning. Night dives were also performed in these months around the third quarter moon phase to observe spawning. In August 2000, gamete release was monitored in aquaria. Four gravid colonies (two female, two male) were located and tagged from the Tynes West site at the Inner Lagoon. Five days after the full moon, five branches approximately 20 cm long from each colony were collected and maintained in aquaria at the outdoor wet bench facilities by the dock of the Bermuda Biological Station for Research. The male and female branches were kept separate in clear plastic containers of static water to retain all spawned gametes. The water was replaced twice daily and aerators supplied the static water with oxygen. The containers were held in a larger aquarium of flowing seawater to reduce the temperature fluctuation.

4.3.5 Fecundity and polyp volume

Male and female fecundity of the tagged colonies prior to spawning each month was determined from the samples collected over 1998-2000 to monitor the month of spawning (section 4.3.4). All spermaries were counted and measured within 10 polyps per branch at 8-10 cm from the branch tip of the male colonies. The oocytes in female colonies were less abundant and so a total of 20 polyps per branch were examined, 10 polyps from 3-5 cm from the branch tip and 10 polyps from 8-10 cm from the branch tip, to examine for intra-branch variation in fecundity (section 4.3.6). The gametes were measured into size classes as defined by Table 4.1. Total oocyte or spermary volume per polyp for each colony was then calculated assuming a sphere of the maximum diameter, d, per size class and using the formula $^4/_3\pi(d/2)^3$. Some spermaries develop slightly oval in shape when mature but were classified as spherical for the purpose of this study. Oocyte volume was further grouped into mature (>500μm), which are those oocytes that are spawned, and immature volume (<100-499μm), which are present in the polyps outside of reproductive months.

Table 4.1: Size classes used in the measuring of *Pseudoplexaura porosa* gametes.

Size class:	A	B	C	D	E	F	G	H
Diameter (microns)	<100	100-199	200-299	300-399	400-499	500-599	600-699	700-800

Variation in polyp size among the tagged *Pseudoplexaura porosa* colonies was examined as a determinant of fecundity. During the summer of 2000, polyp dimensions were measured from two branch samples collected from each tagged colony. The branch width of each sample was measured using callipers at a distance of 3-5 cm and 8-10 cm from the branch tip, as these were the locations at which gamete counts and measurements were made. Cross sections of the branch were then cut at these locations, which resulted in longitudinal sections of the polyps surrounding the central axial rod. The dimensions of 10 randomly selected polyps were measured from the two cross sections per branch, equating to 40 polyps from each tagged colony at all the sites being measured (total of 20 polyps from each of two branches per colony). The polyps are conical in shape and so the dimensions measured were basal diameter (A), mouth diameter (a) and polyp height (H). Polyp volume was estimated using the formula

H[A+a+sqrt(Aa)]/3. The mean polyp volume was then determined for each tagged colony. Variations in mean polyp volume of the colonies between the study sites and the reef zones were examined using one-way nested ANOVA, followed by multiple comparison of means. Prior to statistical analysis, the data were tested for conformation to the parametric assumptions of ANOVA using the Bartlett test for homogeneity of variances (Sokal and Rohlf, 1995) and the Kolmogorov-Smirnov test for normality (Wilkinson *et al.*, 1992).

4.3.6 Intra-branch variation in oocyte fecundity

To explore the possibility of intra-branch variation in oocyte production, all oocyte counts from the female colonies sampled in 1999 and 2000 (section 4.3.5) were made from 10 polyps taken 3-5 cm from the branch tip and 10 polyps from 8-10 cm from the branch tip. Total oocyte volume for each location on the branch of the tagged colonies was calculated for immature oocytes (<500μm) and mature oocytes (>500μm), as defined in section 4.3.5. *P. porosa* gamete volume per polyp is correlated to the polyp volume (section 4.3.5 and 4.4.5). All oocyte volumes were therefore expressed as per polyp volume (mm^3) to account for any inter-colony variation between polyp fecundity. A series of Wilcoxon signed ranks tests (Wilkinson *et al.*, 1992) were performed to examine for any differences in oocyte volume and the position of the polyps from the branch tip. Only the sample dates just prior to spawning in reproductive months in 1999 and 2000 (July and August) were included to assess intra-branch variation in the fecundity of mature oocytes.

4.4 Results

4.4.1 Spicule analysis of tagged colonies

The most common spicules present in all the preparations made from the tagged *Pseudoplexaura porosa* colonies were the colourless spindle (Figure 4.1A and B) and the purple capstans (Figure 4.1B and C). The colourless spindle was as described previously for *P. porosa* with widely spaced tubercles, which is a differentiating feature from the congeneric species *P. flagellosa* and *P. wagenarri*. These spindles were generally greater than 400μm in length and were frequently found in all the spicule preparations (Appendix 4.2). The purple spindles were present from all of the spicule preparations (Appendix 4.2). Similar to their colourless counterpart, these had widely spaced tubercles (Figure 4.1D). A variant between the purple spindle and the capstans is the 3- or 4-rayed purple spindle (Figure 4.1E) that was present from 55% of the total spicule preparations made and 70% of the colonies (30 out of 43; Appendix 4.2). Occurrence among and between the colonies varied from rare to abundant. Colourless rayed spindles were rare in all preparations (not included in the abundance tables), which is as described for *P. porosa*.

The confirming spicules for *P. porosa* are the thorny clubs and the unilateral spiny spindle variant (Figure 4.2). Clubs were present in the majority (95%) of the spicule preparations and always from at least one of the replicate samples taken per colony (Appendix 4.2). All clubs were confirmed to be 'thorny' in shape, lacking the more rounded, foliate ends characteristic of the congeneric species. These 'classic' clubs for *P. porosa* identification are shown in Figure 4.2A-D. The unilateral spiny spindle, as described by Bayer (1961), was rare in the spicule preparations, confirming the results of other studies in Bermuda (A. Neill, J. Gelerter, J. Bilewitch, unpub. data). Gradations between the colourless spindle, the unilateral spiny spindle and the clubs were common and these previously uncharacterised spicules were grouped under the term 'spiny spindle'. A club was defined as being ≤300μm in length with thorny projections that are extensive at one end (Figure 4.2A-D). A spiny spindle was defined from a club as its length being >300μm (Figure 4.2E-G). The closest variant of the

Figure 4.1: *Pseudoplexaura porosa* spicules: abundant spindles and capstans. See Figure 4.3 for clubs and club variants.

Scale bar = 100 μm.

A: Colourless spindle

B: Colourless spindle and purple capstans

C: Purple capstans

D: Purple spindle

E: 4-rayed purple spindle

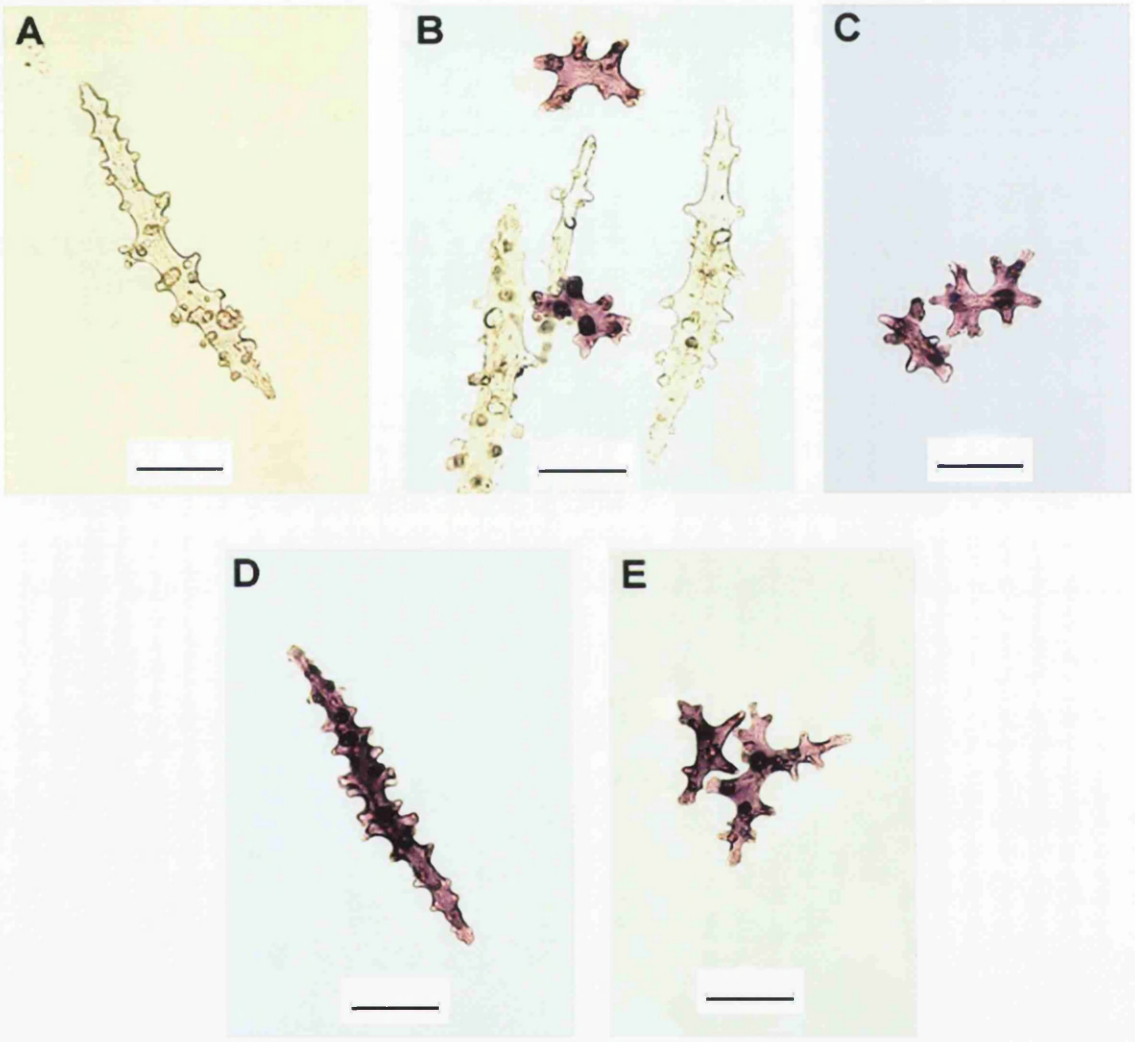
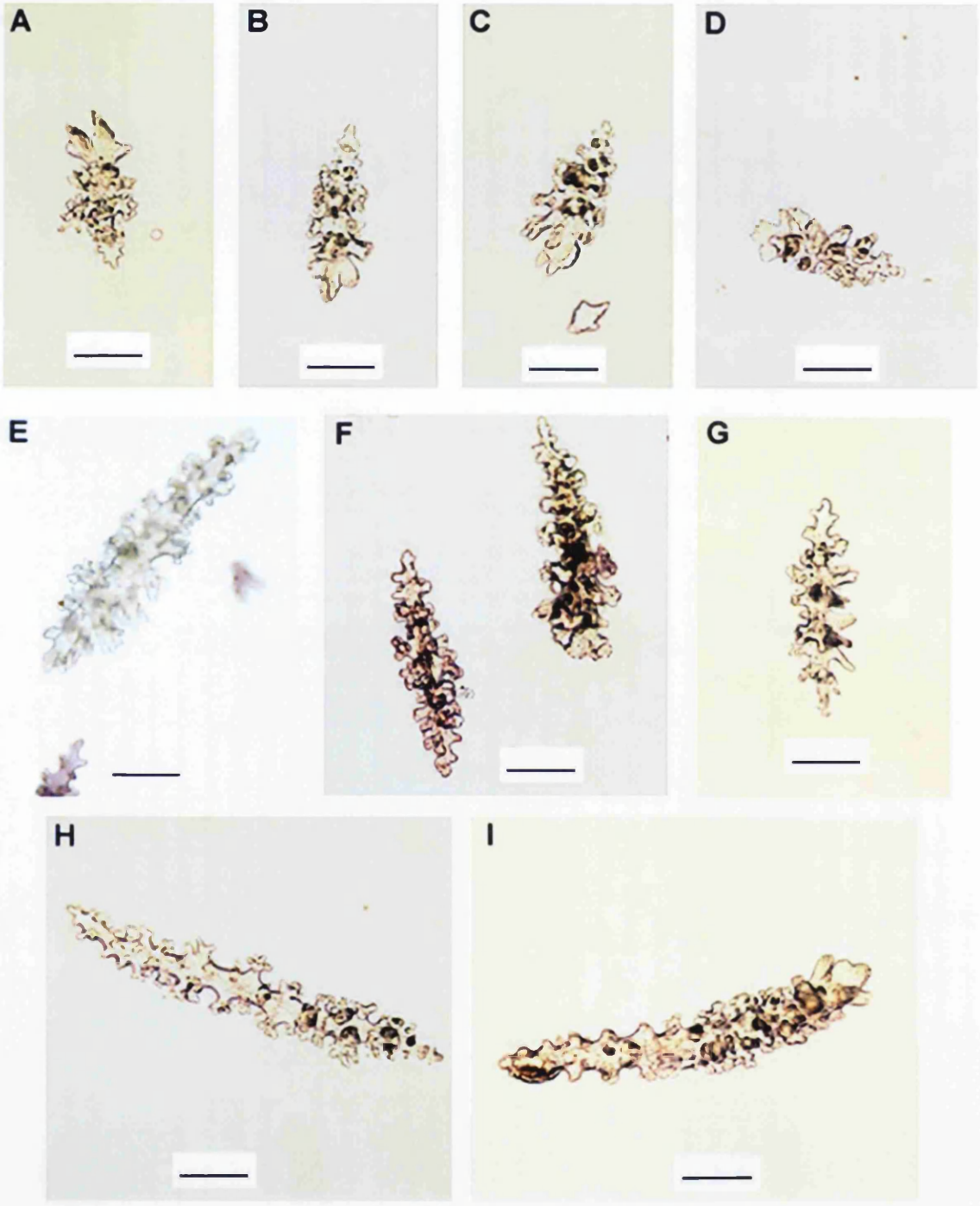

A
B
C
D
E

Figure 4.2: *Pseudoplexaura porosa* spicules: clubs and club variants.
Scale bar = 100 μm.

A-D: 'Classic' clubs, showing the morphological variation
E-G: Small variants of the spiny spindle with club-like projections
H: Variant of the colourless spindle resembling spiny spindle
I: Club-like projections on a spiny spindle


A
B
C
D
E
F
G
H
I

common spindle was 400-700μm in length and was differentiated from the spindle by being thicker with the tubercles more prominent and often forming projections (Figure 4.2H). Club-like projections were sometimes seen at one end of the spiny spindle (Figure 4.2I). There were many variants of the spiny spindle grading in size and shape. Overall, the spicules grouped into the spiny spindle category occurred in 88% of the colonies (38 out of 43) with a 56% occurrence from the total spicule preparations made.

4.4.2 Population Study

A total of 198 *Pseudoplexaura porosa* colonies >50 cm tall were measured and their height determined from the population survey performed across the replicate study sites at the Inner Lagoon, Outer Lagoon and Rim Reef zones of the Bermuda North Lagoon. Distribution of *P. porosa* across these reef zones is not uniform and a greater number of colonies were found at the Inner Lagoon and Rim Reef sites compared to the low coverage at the Outer Lagoon sites (Figure 4.3). Colony density is similar between the replicate reef sites within each zone.

The sex of all *P. porosa* colonies from the population study was determined during peak reproductive months. The sexes are separate at both the polyp and the colony level and developing embryos or larvae were never seen in the polyps. Non-reproductive colonies >50 cm tall accounted for 20% of the total number of colonies surveyed across all the study sites (40/198). The highest percentage of non-reproductive colonies occurred at the replicate sites from the Inner Lagoon reef zone (35% at Tynes West and 21% at Tynes East). Non-reproductive colonies represented a smaller percentage at the Rim Reef zone (19% at Twin Breaker and 14% at Hog Breaker) and the least percentage of immature colonies occurred at the Outer Lagoon reef zone (13% at Crescent B and 6% at Crescent C). The overall sex ratio at the Inner Lagoon is a male:female ratio of 1:1.15 (Figure 4.3). At the Outer Lagoon, where colony density is lowest, the replicate sites vary in the proportion of male to female colonies. There is an even sex ratio at Crescent B (7/7) but the ratio is dominated towards male colonies at Crescent C, being male:female 1:0.33. Female colonies at the Rim Reef zone out

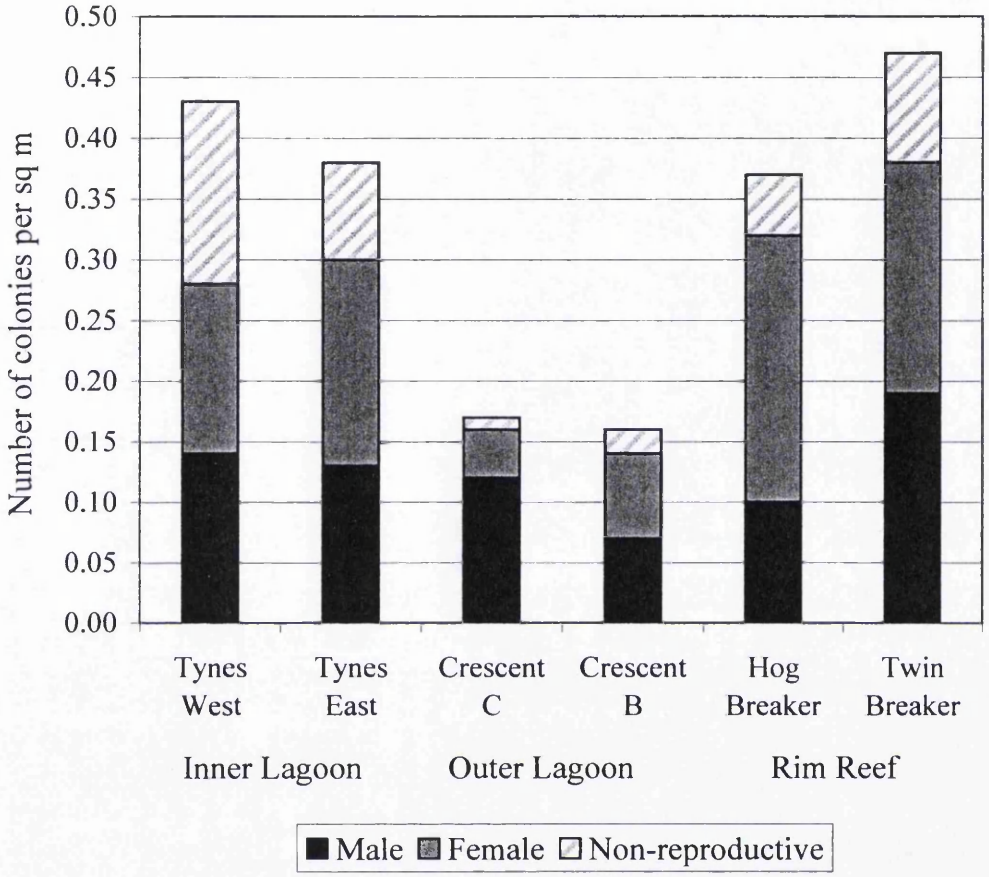


Figure 4.3: Stacked bar chart showing the density and sex ratios of *Pseudoplexaura porosa* colonies >50cm tall surveyed at the replicate study sites of the three reef zones across the Bermuda North Lagoon. An area of 100 sq m was surveyed at each site.

numbered male colonies with an overall sex ratio of 1:1.41 (29/41). There was over double the number of female to male colonies at Hog Breaker of the Rim Reef (10/22), whereas there was an even sex ratio at Twin Breaker (19/19). The overall sex ratio of males to females recorded across all the sites and zones was 1:1.11 (75/83) which was not statistically different from unity (χ^2= 8.65, df= 5; P= 0.124).

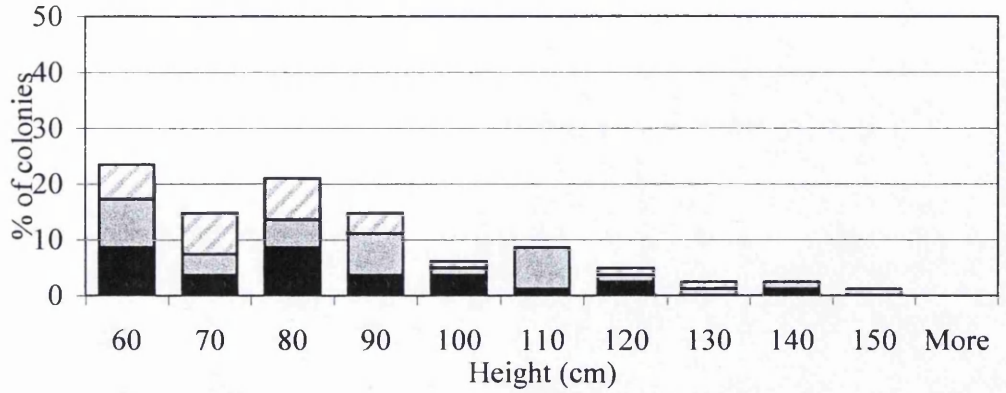
The majority of *P. porosa* colonies >50 cm tall that were measured in this population study at the reef sites were 50-100 cm tall (Figure 4.4). Only 8% of the colonies at the Rim Reef were >90 cm tall, compared to 14% at the Outer Lagoon and 26% at the Inner Lagoon. This greater size range of larger colonies at the Inner Lagoon included male, female and non-reproductive colonies. Non-reproductive colonies >90 cm tall were not found at any of the other reef zones. At the Outer Lagoon, all colonies >100 cm tall were in the 120-130 cm size range and were male. The majority of colonies at the Rim Reef >90 cm tall were male of varying sizes. Only a small number of tall colonies (120-130 cm tall) at this reef zone were found to be female. The tallest colony recorded was from Twin Breaker at the Rim Reef and was a male colony 152 cm tall.

4.4.3 Gamete development

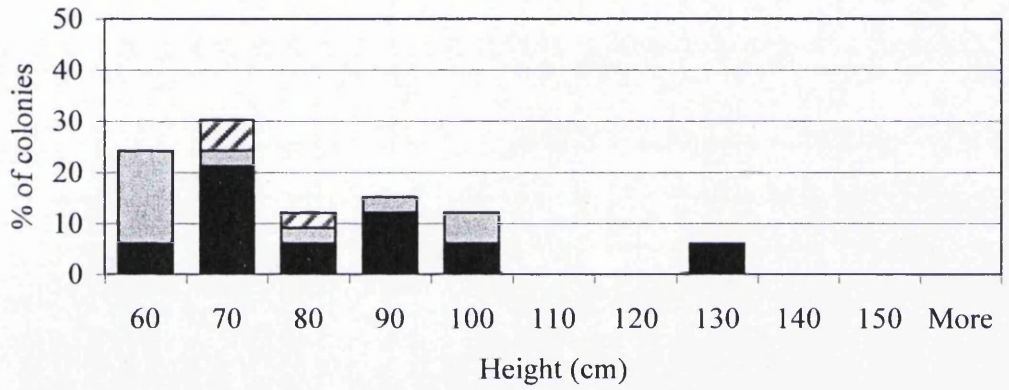
Gamete Location

As with other plexaurid gorgonians, the polyps of *Pseudoplexaura porosa* are embedded at a perpendicular orientation in the coenchyme surrounding the supporting soft, cross-chambered central axis that surrounds the gorgonin axial rod. Cutting a cross-section of a branch results in the longitudinal sectioning of 12-14 polyps (Figure 4.5A). Each polyp has eight pinnate tentacles surrounding an oral disk with a slit-like siphonoglymph leading to a pharynx, which occupies approximately the top third of the polyp. There are eight mesenteries and, as in many other octocorals, two mesenteries, known as the asulcal mesenteries, have strongly flagellated and much convoluted mesenterial filaments (AF in Figure 4.5B). Developing gametes were never seen on these asulcal mesenteries. The developing gametes were located towards the basal end of the other mesenteries and when large they filled the polyps (Figure 4.5).

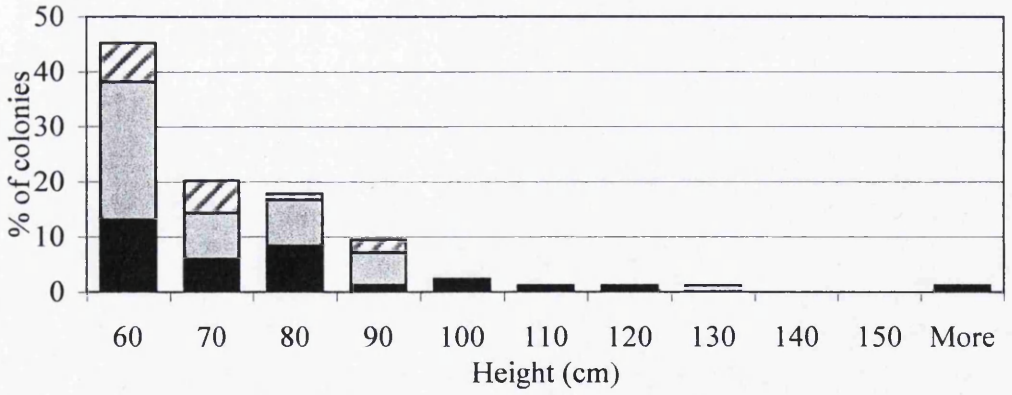
Inner Lagoon (n=81)



Outer Lagoon (n=33)



Rim Reef (n=84)



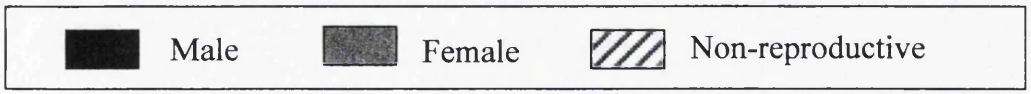


Figure 4.4: Size frequency distribution of *Pseudoplexaura porosa* colonies >50cm tall and their reproductive state. Shown as a percentage of the total number of colonies surveyed within each reef zone (n). An area of 100 sq m was surveyed at each site, with two sites in each zone.

Figure 4.5: Cross sections cut by hand of *Pseudoplexaura porosa* branches before and after spawning. This cut gives a longitudinal section through the polyps that surround the central axis. The tissues were fixed in 9% formalin. Scale bars shown are 400 μm, with the exception of Figure A, which represents 4 mm.

A: Cross section of the branch of a female colony resulting in longitudinal sections through the polyps and tentacles (T) surrounding the central axial rod. Mature oocytes (MO) are seen at the base of the polyp. Scale bar =4 mm

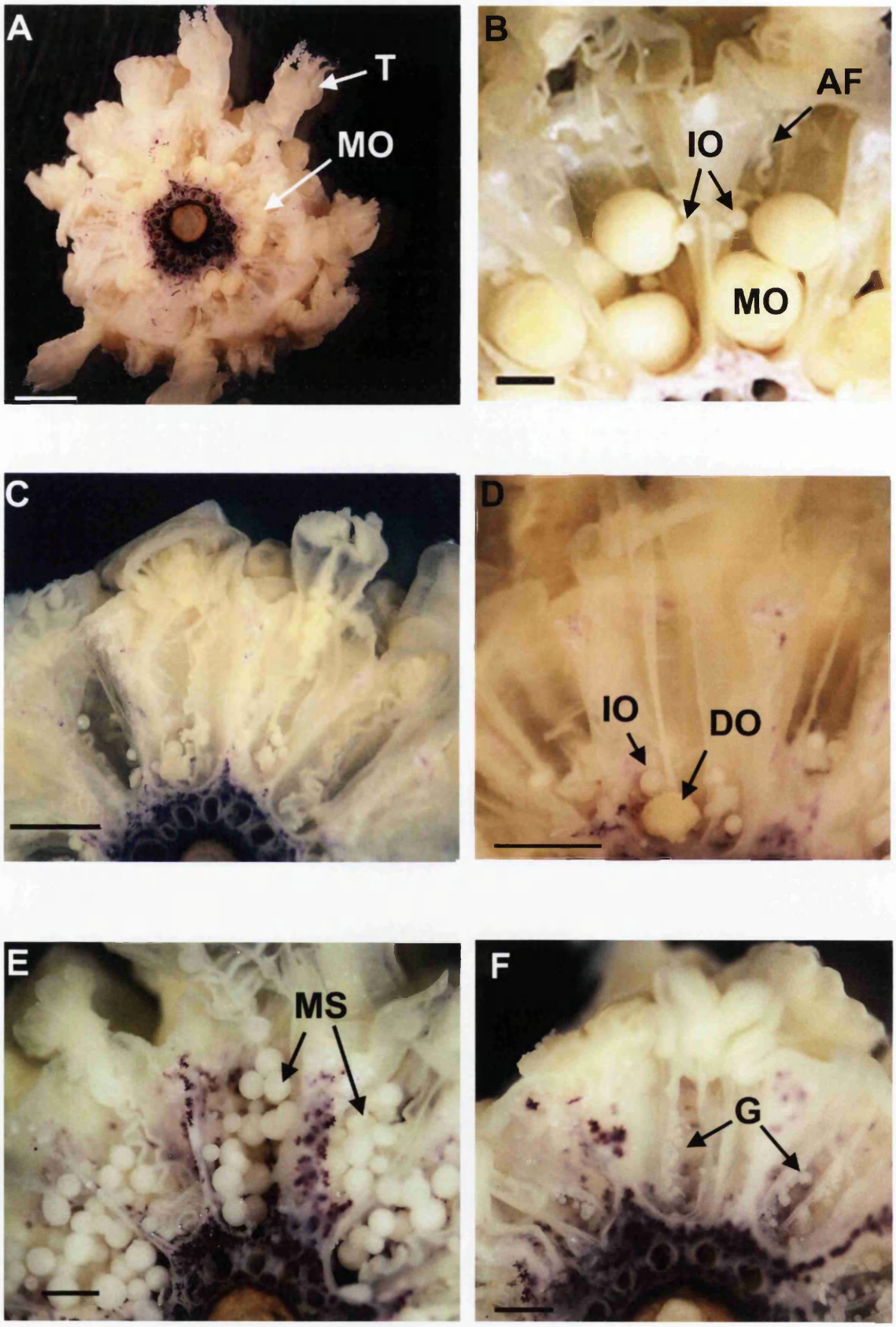
B: Close-up of mature oocytes (MO) next to immature oocytes (IO). The convoluted asulcal filaments (AF) do not possess gametes.

C: The same female colony 1day after spawning was complete.

D: Close-up of a degenerating mature oocyte (DO) left in the colony after spawning, the surface of the oocyte is uneven and shrivelled. The adjacent immature oocytes (IO) are present in the polyps after spawning.

E: Cross section of the branch of a male colony with mature spermaries (MS).

F: The same male colony 1 day after spawning, all mature spermaries are shed and small swellings or 'grapes' (G) are left.


A
T
MO
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MO
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IO
DO
E
MS
F
G

Oocyte Development

The oocytes are clearly visible within the gastrovascular cavity (Figure 4.5A and B) and are attached to the mesenteries by a short mesogleal stalk. Samples taken from female colonies outside of the reproductive months in April 1998 (random colonies), May-June and November 1998 (tagged colonies) and in May-June and September-October in 1999 and 2000 (tagged colonies) all contained small oocytes (<300μm). One cohort develops from this reserve to mature oocytes (500-800μm) during each spawning month in the summer. In samples taken just prior to spawning of these months, there were commonly 3-6 immature oocytes (<500μm) per polyp with a maximum of 11.5 (Figure 4.6A). The larger, mature oocytes were less numerous, with a predominant frequency of less than one oocyte >500μm polyp^{-1} with a maximum of 5.9 and mean of 1.04 oocytes polyp^{-1} (Figure 4.6B).

Wax sections were made to examine oocyte development at the histological level (Figure 4.7). All oocytes above approximately 100μm diameter become surrounded by a layer of endodermis, 10-13μm thick, which develops to approximately 40μm thick as the oocytes grow (G in Figure 4.7B and E). The germinal vesicle and nucleolus are clearly visible, even in the immature oocytes (size classes a-c, Figure 4.7B) and do not appear to increase in size with the oocyte during further oogenesis (germinal vesicle approximately 55-65μm diameter). The germinal vesicle is located at the periphery of the oocytes next to the mesogleal stalk and remains in this position throughout development to maturity (Figure 4.7C, E and F). The position of the germinal vesicle suggests that there may be a trophonema, as occurs in some zoanthids (Ryland, 1997), actiniarians (Larkman and Carter, 1982) and ceriantharians (J.S. Ryland, pers. comm.). This structure is thought to have a nutritive role as endodermal cells extrude through the mesoglea making contact with the oocyte at the site of the germinal vesicle. Examination of this region of the *P. porosa* oocytes at high power, however, did not detect any encroaching of cells across membranes and so there does not appear to be a trophonema. As the oocytes grow in size their ooplasm becomes filled with yolk granules and lipid droplets and the mature oocytes become more granular in appearance. The endodermal layer surrounding the largest oocytes was often detached (Figure 4.7F).

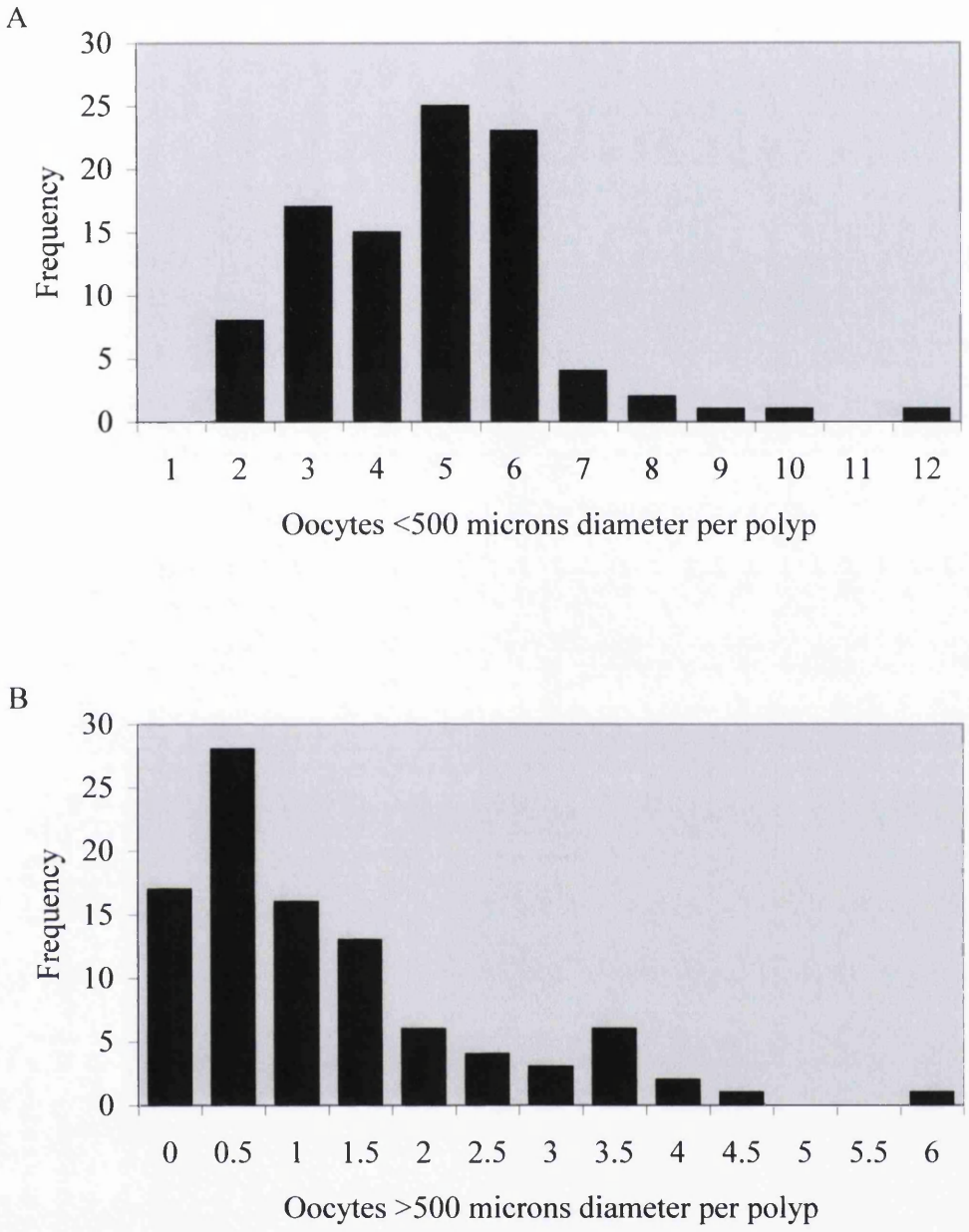
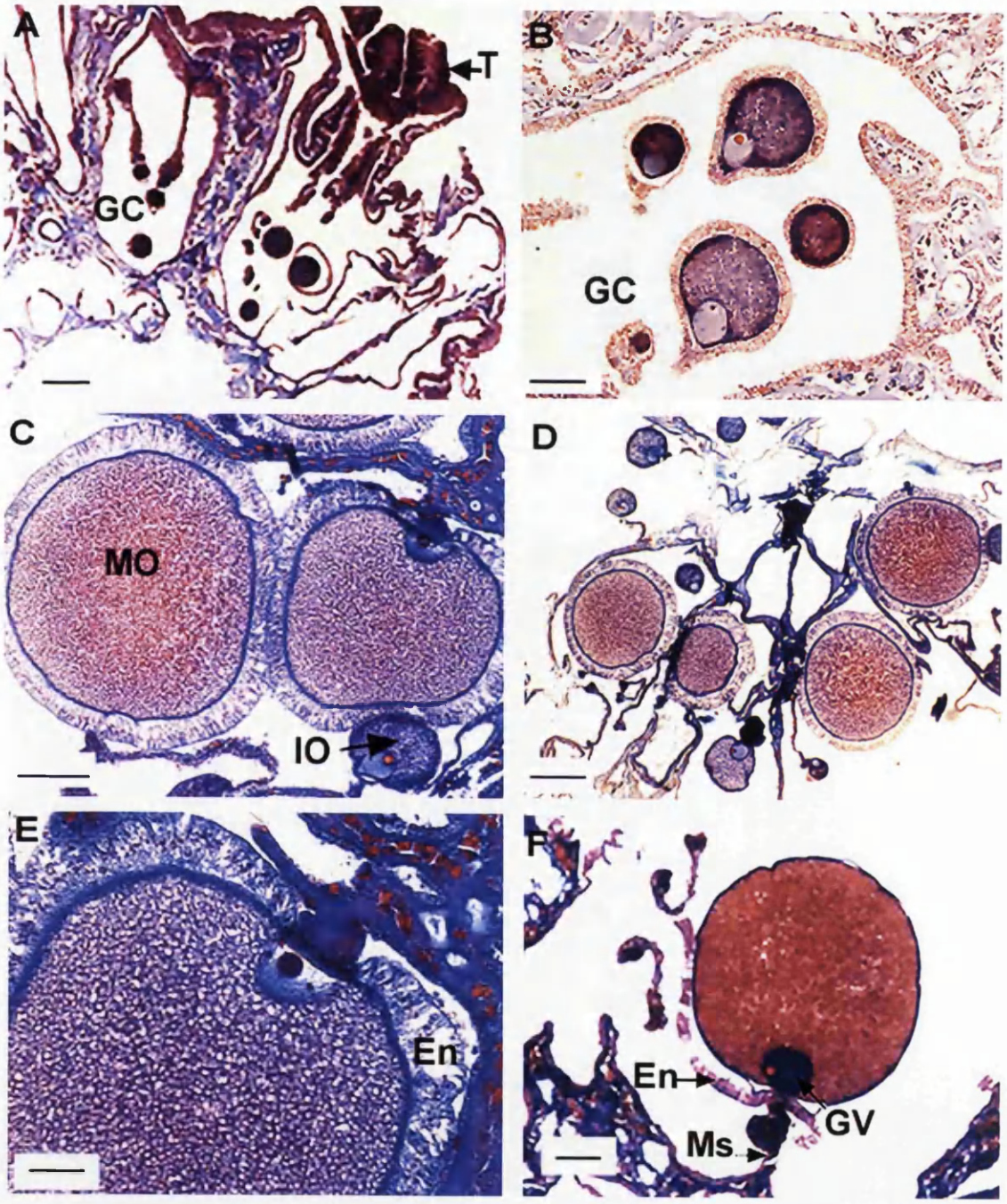


Figure 4.6: Frequency distributions of the number of oocytes per polyp in *Pseudoplexaura porosa* colonies for immature oocytes <500 microns diameter (A); and mature oocytes >500 microns diameter (B). The oocyte frequencies are pooled from samples around the full moon of each spawning month of 1998-2000 from all sites. Total sample size is 97 branches (mean from 20 polyps per branch).

Figure 4.7: Histological slides of developing oocytes in female *Pseudoplexaura porosa* colonies. Tissues were fixed in 9% formalin, decalcified with 10% formic acid and stained with Mallory's Triple Stain.

A: Longitudinal section of a female polyp through the tentacle (T) and gastrovascular cavity (GC). Circular immature oocytes are present within the GC. Scale bar =500 μm.
B: Close-up of immature oocytes at base of polyp inside the gastrovascular cavity (GC). Scale bar =200 μm.
C and D: Mature oocytes (MO) adjacent to immature oocytes (IO). Scale bar for C =200μm, and for D =400 μm.
E: Mature oocyte showing details of the surrounding endodermis (En) and the peripheral location of the germinal vesicle. Scale bar =100 μm.
F: Mature oocyte ready for spawning with the surrounding endodermis (En) dislodged during the histology process. Note the peripheral location of the germinal vesicle (GV) next to the mesogleal stalk (MS). Scale bar =200 μm.


A
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En
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En
GV
Ms

Spermary Development

In male colonies, spermaries were absent for most of the year. Spermaries were first recognisable as small swellings approximately one month prior to spawning and as these started to grow they appeared as 'bunches of grapes' (often <50μm diameter) attached by short stalks to the mesentery. The spermaries rapidly grew and were classified using the same size categories as the oocytes up to size class E, 400-499μm. The spermaries were round when immature but could be differentiated from oocytes by being softer (if poked by a needle the indentation remained). When mature, the majority of spermaries remained round, with a few growing slightly oval in shape (Figure 4.5E). The smaller spermaries were generally more abundant than oocytes, the greatest frequency of polyps containing between 5 and 10 spermaries polyp^{-1}, with a maximum of 49 and mean of 12.32 spermaries polyp^{-1} (Figure 4.8).

The histological sections revealed that the young spermary is filled with large spermatocytes (3-4μm diameter) with darkly staining nuclei, which surround the spermary wall and leave a central lumen (Figure 4.9B). A layer of endodermis also surrounds the spermaries. In contrast to the developing oocyte, this layer is thickest surrounding immature spermaries (10-15μm) and becomes thinner as the spermary matures (7-9μm). As the spermaries increase in size, the spermatocytes become more numerous and tightly packed around the persisting central lumen (Figure 4.9C). The large spermaries fill the gastrovascular cavity (Figure 4.9D). Mature spermaries just prior to spawning were packed with smaller spermatids (2-3μm) and spermatozoa with well developed tails (Figure 4.9E and F).

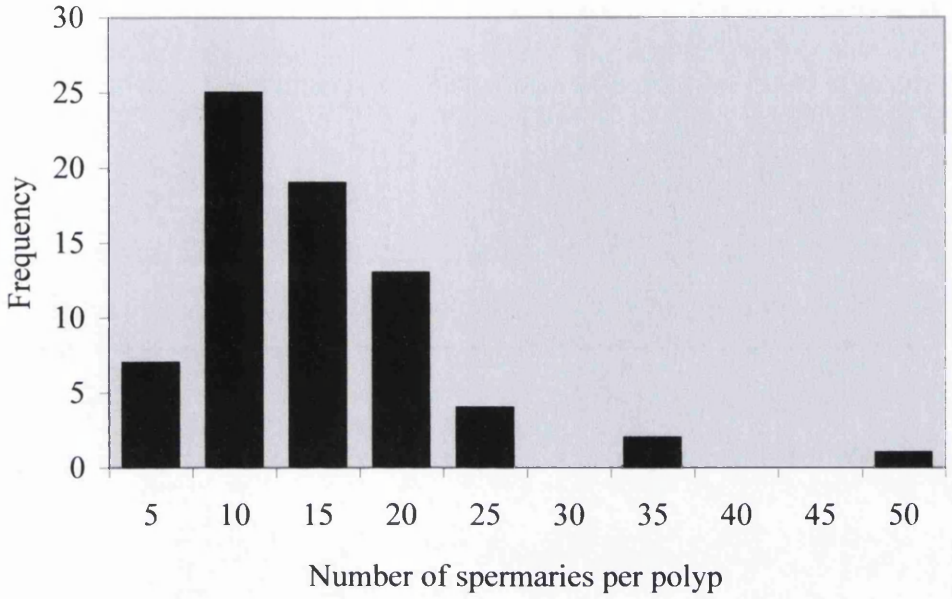


Figure 4.8: Frequency distribution of the number of spermaries per polyp in *Pseudoplexaura porosa* colonies. The spermary frequencies are pooled from samples around the full moon of each spawning month of 1998-2000 from all sites. Total sample size is 71 branches (mean from 10 polyps per branch).

Figure 4.9: Histological slides of developing spermaries in male *Pseudoplexaura porosa* colonies. Tissues were fixed in 9% formalin, decalcified with 10% formic acid and stained with Mallory's Triple Stain.

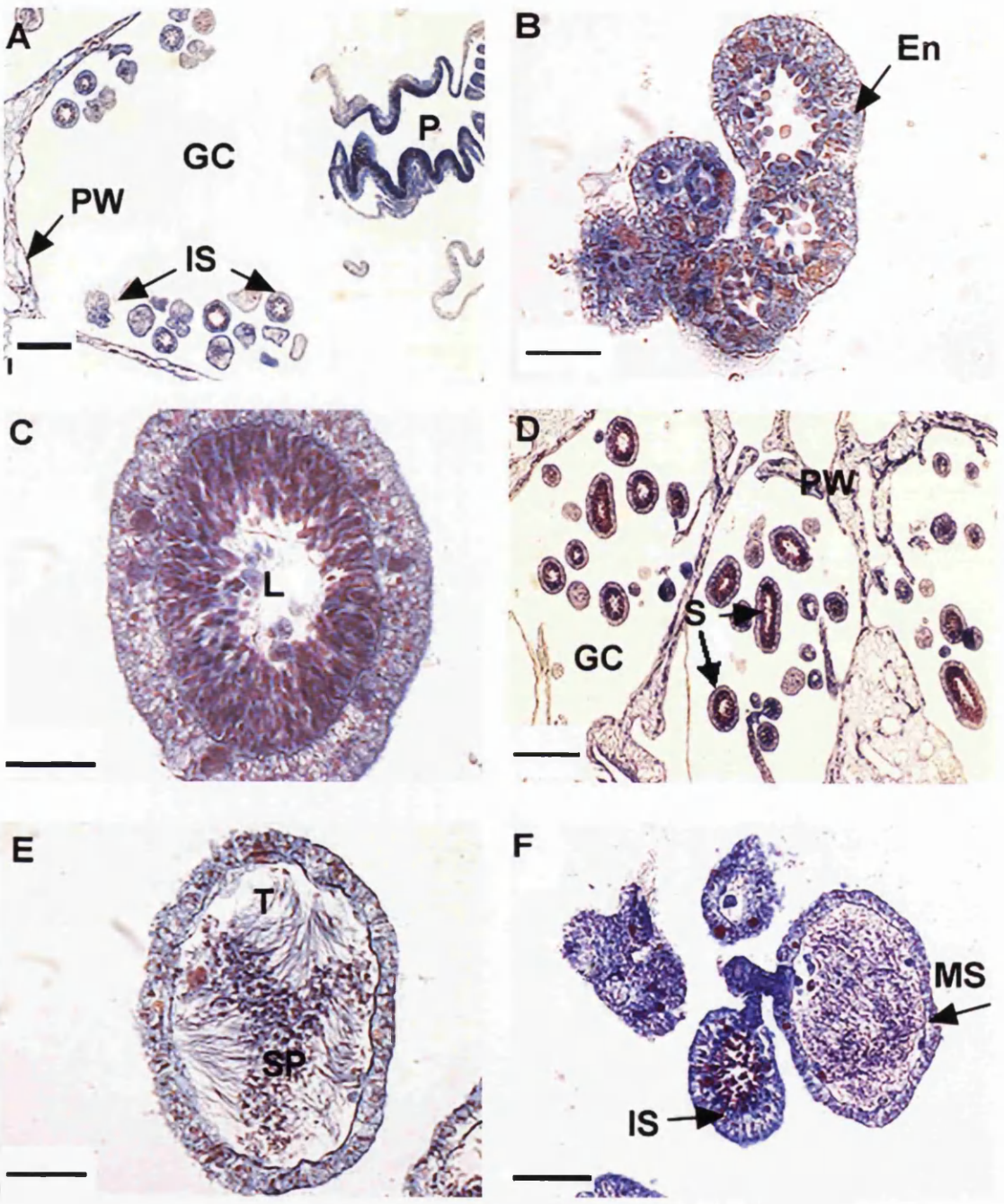
A: Longitudinal section of a male polyp through the pharynx (P) and the gastrovascular cavity (GC) with immature spermaries (IS) next to the polyp wall (PW). Scale bar =500 μm.

B and C: Close-up of immature spermary with thick endodermis (En), peripheral spermatocytes and central lumen (L). Scale bar =100 μm.

D: Longitudinal section of a male polyp with many maturing spermaries (S) with spermatocytes and central lumens, within the gastrovascular cavity (GC). Scale bar =500 μm.

E: Mature spermary with spermatids (SP) and spermatozoa with tails (T). Scale bar =200 μm.

F: Mature spermary (MS) next to an immature spermary (IS) with spermatocytes. Scale bar =200 μm.


A
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4.4.4 Spawning

Months of spawning

The occurrence of spawning was inferred from the disappearance of mature oocytes and spermaries from tagged colonies, which were regularly monitored by hand sections (Figure 4.5C). During spawning, the majority of mature oocytes of diameter exceeding approximately 500 μm were released, leaving the immature oocytes that are always present in the polyps. A few mature oocytes sometimes remained in the polyps and within days were seen degenerating (Figure 4.5D) and were presumably resorbed, as they were not found in the colonies a few days later. Samples collected from male colonies immediately after a spawning event indicated that sperm release occurred from all of the developed spermaries. The ruptured spermary sacks were either discharged during spawning or were resorbed, as they were not seen on consecutive sample dates. If there was a successive spawning event, 'grapes' and 'swellings' (100-200μm diameter) remained in the polyps (Figure 4.5F) that were the next cohort that rapidly grew to maturation the following month. Once spawning was complete for a season, no spermaries were seen remaining on the mesenteries.

Mature gametes were present in the colonies, thereby indicating the potential for spawning around full moons from August until October in 1998 and during July and August in 1999 and 2000 (Table 4.2). In 1998, mature gametes were present in the sampled colonies from the Outer Lagoon and Rim Reef sites (the Inner Lagoon sites were not sampled in 1998) between August and October. The duration of spawning was variable among the colonies. Mature oocytes were only present in the female colonies at the Outer Lagoon for one spawning month in September (Table 4.2). Mature spermaries were present in two of the three monitored male colonies in August and then all male colonies spawned in September. There were no mature gametes in the female or male colonies at the Outer Lagoon in October 1998. All colonies from the Rim Reef spawned in August and September 1998 (Table 4.2). In contrast to the Outer Lagoon colonies, mature gametes persisted in small numbers into October from two of the three female colonies and one of the two male colonies from the Rim Reef for a third month

Table 4.2: The number of *Pseudoplexaura porosa* colonies from the study sites that spawned each month over 1998, 1999 and 2000. Spawning was inferred from the presence of mature gametes around the full moon of each month. Sample size n is the number of colonies monitored at each site. New colonies were tagged in 1999 and sampled in addition to all tagged colonies from 1998. Some colonies were not re-sampled in 2000 (see text for details). The total number of male and female colonies sampled each year and the total number that spawned in each month are shown at the base of each table.

Year: 1998		Sample n		July		August		September		**October**	
Reef Zone	Site	F	M	F	M	F	M	F	M	F	M
Outer Lagoon	CC	2	3	0	0	0	2	2	3	0	0
	CB	No sample		-	-	-	-	-	-	-	-
Rim Reef	HB	3	2	0	0	3	2	3	2	2	1
	TB	No sample		-	-	-	-	-	-	-	-
Inner Lagoon	TW	No sample		-	-	-	-	-	-	-	-
	TE	No sample		-	-	-	-	-	-	-	-
	Total	5	5	0	0	3	4	5	5	2	1
Year: 1999		Sample n		July		August		**September**		**October**	
Reef Zone	Site	F	M	F	M	F	M	F	M	F	M
Outer Lagoon	CC	4	3	3*	3	3	3	0	0	0	0
	CB	4	2	-	2	4	2	0	0	0	0
Rim Reef	HB	5	3	4	3	5	3	0	0	0	0
	TB	3	4	1*	4	3	4	0	0	0	0
Inner Lagoon	TW	5	3	5	3	5	3	0	0	0	0
	TE	4	3	4	3	4	3	0	0	0	0
	Total	25	18	17	18	24	18	0	0	0	0
Year: 2000		Sample n		July		August		September		October	
Reef Zone	Site	F	M	F	M	F	M	F	M	F	M
Outer Lagoon	CC	3	2	3	0	3	2	0	0	0	0
	CB	4	2	3	2	2	2	0	0	0	0
Rim Reef	HB	5	2	2*	1	3	1	0	0	0	0
	TB	3	3	3	3	2	3	0	0	0	0
Inner Lagoon	TW	4	2	2	1	2	2	0	0	0	0
	TE	3	3	3	1	3	3	0	0	0	0
	Total	22	14	16	8	15	13	0	0	0	0

* one female colony not sampled
- No sample was collected

of spawning (Table 4.2). No oocytes or spermaries were found in the Rim Reef colonies at the end of October.

Spawning of all tagged colonies was earlier in 1999 than in to the previous year, and took place in July and August (Table 4.2). All male colonies across the three reef zones (Inner Lagoon included in 1999) spawned in July and August 1999, as large spermaries filled the polyps. All but one female colony at Crescent C of the Outer Lagoon spawned in August 1999, but only 18 of the 22 sampled female colonies across the reef zones spawned in July 1999 (no samples from Crescent B). One colony from Hog Breaker and two colonies from Twin Breaker only developed mature oocytes in August 1999. There were no mature gametes in the colonies from any of the reef zones in September or October 1999.

With the exception of Crescent B at the Outer Lagoon, between one and two colonies from the other sites were not re-sampled for gamete development over the summer of 2000. Two colonies at Crescent C appeared unhealthy with the degeneration of some branch areas and the presence of large quantities of mucus. This condition was not particular to these colonies and was observed from random *P. porosa* colonies at the Outer Lagoon and Rim Reef in this summer and in other years. Two of the larger colonies at the Inner Lagoon sites and one colony from Twin Breaker had fallen over, presumably being dislodged during the winter storms. It was decided not to re-sample one male colony at Tynes East of the Inner Lagoon as three colonies were adequate, given the time constraints. The tag from one colony at Hog Breaker was lost and the colony was not re-located. Spawning of the monitored colonies took place over July and August in 2000, the months in which gamete maturation had occurred in 1999 (Table 4.2). In contrast to the previous year, some male colonies did not spawn over both months in 2000 (8 out of 14 colonies spawned for two months). Spawning occurred from 16 female colonies in July 2000 and 15 female colonies in August 2000 (out of 22 sampled colonies). Of these colonies, 14 contained mature oocytes over both spawning months. Five female colonies (two from the Inner Lagoon and Rim Reef and one from the Outer Lagoon) and one male colony (from the Rim Reef) did not develop mature gametes over the summer of 2000, although these colonies were reproductive the previous year.

The timing of spawning

The collection of branches of the tagged *Pseudoplexaura porosa* colonies on frequent dates over the spawning months in 1998 confirmed that mature gametes were present around the full moon. To determine the exact day of spawning, ten branches were collected from additionally tagged colonies from the Inner Lagoon (five colonies from Tynes West and five colonies from Tynes East) and examined for mature gametes around the full moons of July and August 1999. Colonies from the Inner Lagoon were also observed *in situ* over these months for spawning activity during night dives. Large gametes were present from nine of the ten additionally tagged colonies four days after the July full moon. A night dive six days after the July full moon saw spawning from large colonies. There were no mature gametes in the ten additionally tagged colonies in a sample collected ten days after the full moon. In August 1999, a night dive was performed on the fourth and fifth night after the full moon and no spawning was observed. Samples were collected from the ten additionally tagged colonies six days after the full moon and large gametes were present from seven. Weather conditions did not permit any further night dives or collections until 13 days after the full moon when a sample collection confirmed that the mature gametes had been shed from all the previously gravid tagged colonies.

The first *P. porosa* colony observed spawning *in situ* at the Inner Lagoon six days after the July full moon in 1999 was at 8.50pm, which was 35 minutes after sunset. Large eggs were observed being 'squeezed' out of the polyps, remaining momentarily around the polyp mouths where they were clearly visible as white spheres along the branches. When the colony branch was moved by water motion the eggs were dislodged into the water column in a synchronous mass. Egg release from an individual colony began at a slow rate for about 10-20 minutes and then increased over the next 15 minutes to a peak in release. There were inter-branch differences in the number of eggs released, varying from whole branches releasing copious amounts of eggs to the sporadic release of a few eggs from other branches. Egg release then declined rapidly and spawning from individual colonies was approximated to take 30-40 minutes with the majority of eggs being released over a 5-10 minute period. Most colonies monitored on the night dive spawned between 9.00 and 10.15pm (45 minutes and two hours after sunset) with the

last colony observed to end spawning around 10.30pm. Sperm release was not seen in the field.

Gamete release was also observed in aquaria. As defined in the material and methods section, five branches from each of two female and two male colonies from the Inner Lagoon were collected in August 2000, five days after the full moon and maintained in outdoor aquaria. Spawning was observed from the same branches on successive nights. Egg release began on the first night at 8.10pm, which was 15 minutes after sunset, and around 20 minutes earlier than the observed spawning time *in situ* (relative to the time of sunset). The duration of spawning was inconsistent between the branches varying between 20 minutes up to one hour. One branch from each of the two female colonies did not release any eggs on the first night. Spawning occurred from all branches on the second night, six days after the full moon. Egg release began earlier, around five minutes before sunset, however but this may be a stress response. The spawning duration was shorter than the previous night, ranging from only ten minutes to 45 minutes and egg release was more intense with a greater number of eggs shed. Egg release was also observed seven and eight days after the full moon from some branches but was sporadic and at erratic times around sunset. A few eggs were seen in the tanks the morning of nine days following the full moon but the study was terminated due to stress to the colony branches. Released oocytes in aquaria ranged from 550-860μm in diameter with a mean of 729 μm (standard deviation ± 87, n=100). The oocytes were white in colour indicating that they did not contain zooxanthellae, as previously recorded for *P. porosa* oocytes by Coffroth and Mulawka (1995).

The aquarium water holding the male colony branches became cloudy, from which sperm release was inferred. The actual release of sperm was not as easily observed but could occasionally be seen in the still water as a stream being expelled from the polyps. Sperm release also occurred on the first night after collection, five days after the full moon and was noticed ten minutes later then egg release at 25 minutes after sunset. Sperm release appeared to last between 45 minutes to one hour. The male branches also spawned six days after the full moon beginning around five minutes after sunset, a delay of ten minutes after the female branches. The intensity and duration of spawning appeared similar to the night before. Sperm release did occur seven days after the full

moon but was sporadic and erratic, comparable to the egg release. No sperm release was seen eight days after the full moon.

4.4.5 Fecundity and polyp volume

The mean polyp volume of the *Pseudoplexaura porosa* colonies was consistently greater at the Inner Lagoon study sites than at the Outer Lagoon and Rim Reef zones, where the smaller polyp volume varied between the replicate study sites (Figure 4.10). The data were analysed using one-way nested ANOVA (Table 4.3), followed by multiple comparison of means (Appendix 4.4).

Table 4.3: One-way nested ANOVA to test for differences between mean polyp volumes of *P. porosa* colonies at replicate reef sites within three reef zones: the Inner Lagoon, Outer Lagoon and Rim Reef. Prior to ANOVA the data were confirmed to be normally distributed and have homogeneous variances (Appendix 4.3).

Level	SS	df	MS	F	P
Zone	532.74	2	266.37	3.81	0.150
Reef within zone	209.58	3	69.86	7.96	0.114
Error	702.32	80	8.78		

The mean polyp volumes of *P. porosa* colonies were not significantly different either within or between the colonies at the three reef zones ($P>0.05$, Table 4.3). The post-hoc test (Appendix 4.4) revealed that the mean polyp volume of the Hog Breaker colonies was significantly smaller to all other means (Figure 4.10). The mean polyp volume of the Twin Breaker colonies was significantly smaller than the Tynes West Inner Lagoon site. Finally, the mean polyp volume of the Crescent C site was significantly different to that of the Inner Lagoon colonies.

There is a positive relationship between total gamete volume per polyp over the reproductive months and the mean polyp volume for female and male *P. porosa* colonies (Figure 4.11). Prior to testing the relationships with correlation statistics, arc

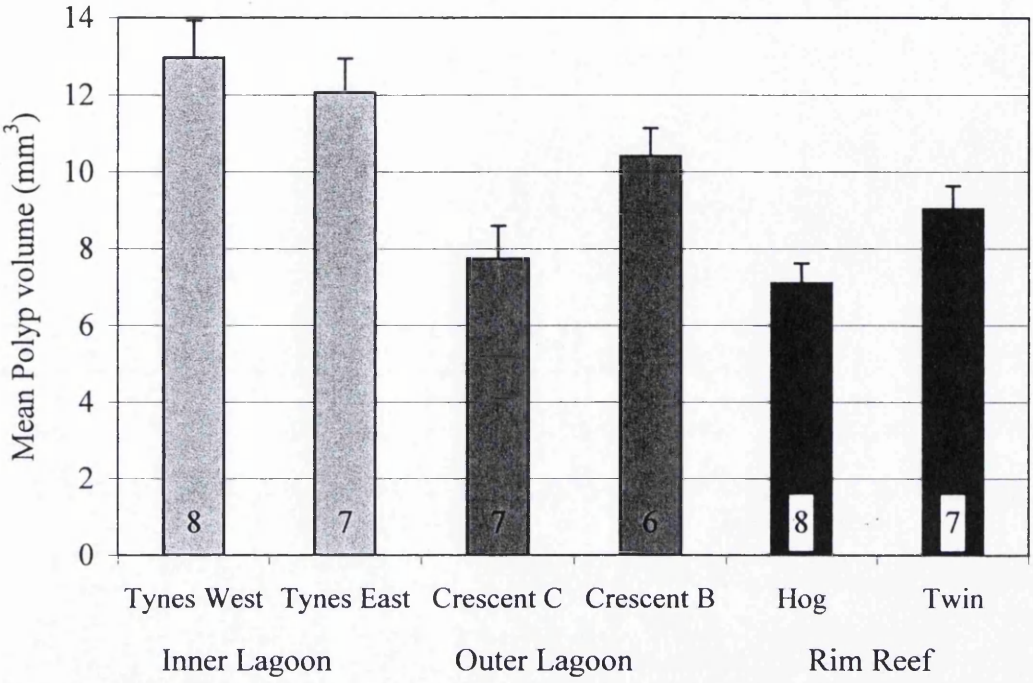


Figure 4.10: *Pseudoplexaura porosa* polyp volume variation at the study sites. The number of colonies from which the mean volume is calculated is shown at the base of each column. A total of 20 polyps were measured from each of two branches per colony (n polyps per colony = 40). Error bars are +1SD

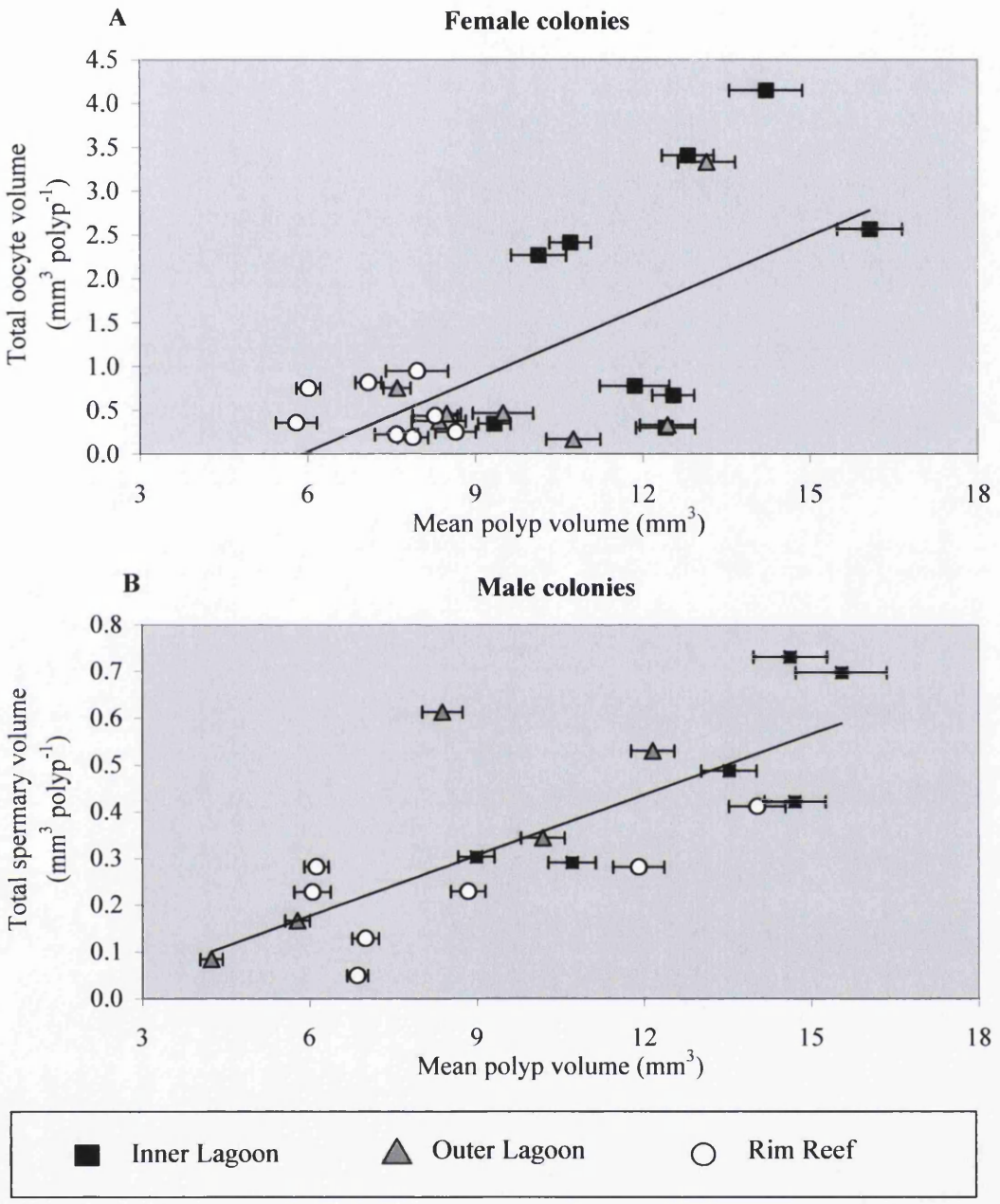


Figure 4.11: The relationship between total gamete volume per polyp and the mean polyp volume of *Pseudoplexaura porosa* colonies. Total oocyte (A) and spermary (B) volume per polyp is shown for each colony combined from the sample dates just prior to spawning in July and August 1999 and 2000. Oocyte volume is of mature oocytes (>500microns) only. The error bars are standard errors. Trendlines are linear regressions on non-transformed data (see text for significance testing on transformed data).

sine transformation of the mean oocyte and spermary volumes were necessary to obtain normality (Appendix 4.5). There was a significant relationship between total gamete volume per polyp and the mean polyp volume for female colonies (product-moment correlation, P= 0.002, r= 0.589, n= 24) and for male colonies (product-moment correlation, P= 0.0001, r= 0.781, n= 18).

4.4.6 Intra-branch variation in oocyte fecundity

Within-branch variability in fecundity was examined among the female tagged *Pseudoplexaura porosa* colonies. All individual samples taken from the tagged colonies at each reef site just prior to spawning in reproductive months in 1999 and 2000 (July and August) were combined and oocyte volume variation at the two branch locations examined using Wilcoxon signed ranks tests. There was no significant difference in the total oocyte volume per polyp at each branch location (3-5 cm and 8-10 cm from the branch tip) for mature oocytes >500μm (P= 0.067, n= 87), or immature oocytes <500μm (P= 0.330, n= 87). The mean total oocyte volume >500μm per polyp at each branch location was then calculated for each reef site prior to spawning of the reproductive months. The results show minimal variation between mean fecundity and the polyp position along the branch from all the study sites prior to spawning of the reproductive months (Figure 4.12). Fecundity is variable between the spawning months with mean oocyte volume greatest in August 1999 (Figure 4.12). Statistical tests were therefore performed to determine whether intra-branch variability in oocyte volume occurred by grouping the data from all colonies for a given month. There were no significant differences between the position from the branch tip and total oocyte volume >500μm per polyp for each of the reproductive months (Wilcoxon signed ranks test: July 99 P= 0.501, August 99 P= 0.144, July 00 P= 0.278, August 00 P= 0.156).

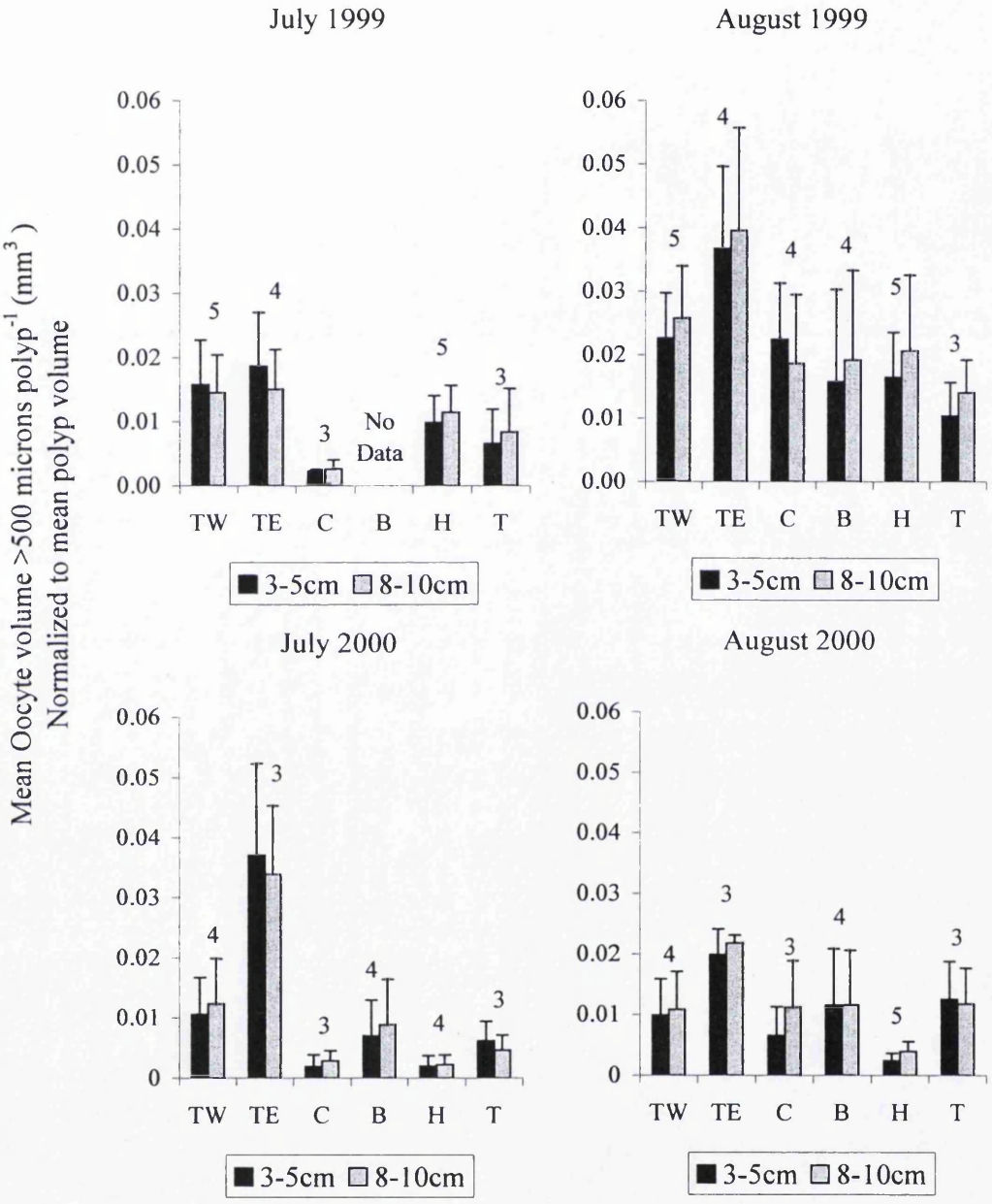


Figure 4.12: Variation in the volume of *Pseudoplexaura porosa* oocytes >500 microns at 3-5cm and 8-10cm from the branch tip, shown for each reef site on the sample date prior to spawning of the reproductive months of July and August 1999 and 2000. Error bars are +1 SE from the mean (number of colonies shown above the bar).

4.5 Discussion

4.5.1 Spicule analysis of tagged colonies

The results of the spicule analysis of the *Pseudoplexaura porosa* tagged colonies endorsed the studies of A. Neill, J. Gelerter and J. Bilewitch (unpublished data) showing that the published description of the spicule morphology of this species by Bayer (1961) is not conclusive. Bayer (1961) stated the presence of the unilaterally spiny spindle as common in *P. porosa* colonies. However, the unpublished data by A. Neill, J. Gerlerter and J. Bilewitch, and the results from the present study, all note the rarity of the unilaterally spiny spindle in Bermuda colonies. Indeed, this characteristic spicule is actually absent from a proportion of the spicule preparations studied. It was originally proposed that colonies lacking the unilaterally spiny spindle represented a sub-species of *P. porosa* in Bermuda (A. Neill, J. Gerlerter). However, Bayer (1961) did note that spicules described as gradations between the unilaterally spiny spindle and the spiny clubs occur, which are similar in appearance to the spicules grouped as spiny spindles in this study. The earlier unpublished data (A. Neill, J. Gerlerter) also contains reference to spicule types similar to the presently described spiny spindles that were then termed "unknown spicules" and "abbreviated clubs". It appears, therefore, that variations at least of the unilaterally spiny spindle were present in previous spicule studies on *P. porosa* colonies in Bermuda, and in the present study. From these results, the distinguishing spicules for *P. porosa* in Bermuda are revised to be the thorny clubs, and gradations between these clubs and the unilaterally spiny spindle commonly occur. The shape and size of these variants of the unilaterally spiny spindle and clubs were inconsistent between the colonies, hence all spicules within the range were termed 'spiny spindles', and this term replaces the 'unilaterally spiny spindle' of Bayer (1961) as a characteristic spicule for *P. porosa*. Clubs and spindle variants of assorted sizes and shapes are also present in the congeneric species *P. flagellosa* and *P. wagenaari*. The diagnostic feature of *P. porosa* clubs and spiny spindles is the pointed and thorny nature of the projections, compared to the foliate and rounded ends to the projections of *P. flagellosa* and *P. wagenaari* (Bayer, 1961).

The spicule analysis of the tagged *P. porosa* colonies confirmed a high variability of spicule types both within and between the colonies. Phenotypic plasticity is common in many clonal invertebrates, including the skeletal features of scleractinian corals (Brakel, 1977; Foster, 1979; Foster, 1980; West *et al.*, 1993), and the nematocysts of zoanthids (J.S. Ryland, unpub. data). Thus, it is not surprising that the spicule morphology of gorgonian corals is also inconsistent among colonies. Some of the spicule types, such as the smaller clubs that possess fewer projections and the shorter common spindles are analogous among the three species of the genus *Pseudoplexaura* (Bayer, 1961). Genetic clarification is needed to define and separate to species level. For the purpose of the present study, all tagged colonies monitored for gamete development and the timing of spawning had similar spicule morphology and possess distinguishing features for *P. porosa*.

4.5.2 Population study

The separate sexes of *Pseudoplexaura porosa* colonies complements the finding to date that all studied gorgonian species are gonochoric (see review, chapter 1), which is in contrast to the dominance of hermaphroditism in scleractinian corals (see review by Richmond and Hunter, 1990; Chapter 1). Whether adopting a brooding or broadcasting mode of reproduction, the majority of gorgonian species studied have an even sex ratio (Vighi, 1970 cited from Coma *et. al.*, 1995; Goldberg and Hamilton, 1974; Grigg, 1977; Coma *et al.*, 1995b; Kapela and Lasker, 1999). The sex ratio of the *P. porosa* colonies in this study varied between the study sites. The patchy occurrence of one sex may be caused by local factors favouring fragmentation, which is a common procedure in many gorgonian species (Lasker, 1983; Walker and Bull, 1983; Lasker, 1984). The overall *P. porosa* colony sex ratio of 1:1.1 male to female in this study, however, was not significantly different from unity, suggesting that the population exhibits an even sex ratio or the sample size did not detect any bias in colony sex.

The only exception to the predominance of an even sex ratio in the gorgonian species studied so far occurs for *Briareum asbestinum* in which the populations in Panama, Puerto Rico and the Bahamas were all male dominated, with an overall sex ratio of 2.2:1 (Brazeau and Lasker, 1990). Fertilisation rates are low in this species and there is

a positive relationship between embryo production in female colonies and nearby male densities (Brazeau and Lasker, 1992). Increasing the density of male colonies is an adaptation to enhance fertilisation success (Brazeau and Lasker, 1990). *P. porosa* has a consistently even sex ratio (in Panama Lasker *et al.*, 1996; Coma and Lasker, 1997a; Kapela and Lasker, 1999; in this study), however, this species experiences a relatively high rate of *in situ* fertilisation with little sperm limitation on the nights when most of the colonies synchronously spawn (Coma and Lasker, 1997a). Thus, the effect of the sex ratio of gonochoric colonies on fertilisation success is species specific and gamete mixing is also influenced by other factors. *Plexaura kuna* co-occurs with *P. porosa* in Panama and also has an even sex ratio (Lasker *et al.*, 1996; Coma and Lasker, 1997b). This species has low *in situ* fertilisation success (Lasker *et al.*, 1996; Coma and Lasker, 1997b) and the fertilisation of eggs is unlikely within 1 m downstream of a colony (Lasker and Stewart, 1992). The greater fertilisation success of *P. porosa* over *P. kuna* has been attributed to the larger sperm densities and egg sizes of the former, as well as larger colonies, increasing the total number of gametes released (Coma and Lasker, 1997; Kapela and Lasker, 1999). In both species there was temporal and spatial variation in fertilisation success (ranging from 0 to 100%), which was caused by environmental factors such as flow rates and biotic factors such as variation in sperm release (Coma and Lasker, 1997b).

There is a reduction in the number of *P. porosa* colonies per sq m at the Outer Lagoon zone compared to the higher colony densities at the Inner Lagoon and Rim Reef zones. In contrast, Smith *et al.* (1984) found no detectable pattern of abundance of gorgonian corals within or between reef zones. Thus, the lower numbers of colonies at the Outer Lagoon may be particular to the two reef sites studied. A more extensive survey is needed to determine the extent that *P. porosa* colony distribution is patchy or whether there is actually a distinct difference in colony density among the reef zones. There was an abundance of tall colonies (>90 cm tall) at the Inner Lagoon reef zone. The growth rate of scleractinian coral species is higher at the inshore reefs of Bermuda than offshore (Dodge and Vaisnys, 1977; Logan and Tomascik, 1991), a consequence of decreased wave energy inshore as well as an increased food availability from the higher productivity closer to land (from Beers and Herman, 1969; Morris *et al.*, 1977). Growth rates of gorgonian species in Bermuda have not been studied. However, the taller colonies inshore are consistent with the observed differences in colony growth form

dependent on location (Smith *et al.*, 1984; pers. obsv.). *P. porosa* colonies from the Inner Lagoon zone, enclosed basins and sheltered inlets are generally slender in form with fewer branches compared to the 'bushier' and shorter growth form of colonies growing offshore and from higher energy environments. Brazeau and Lasker (1988) also correlated increasing wave energy with a decrease in branch length and increase in the number of branches of plexaurid species in Panama.

The studied *P. porosa* colonies in Panama were estimated first reproductive at 50 cm tall (Kapela and Lasker, 1999). This is larger than in some other plexaurid gorgonians (see Chapter 1), although it is potentially at the same age on account of the fast growth rate of the species. Height at first reproduction was not examined in this study, although the majority (80%) of colonies >50 cm tall sampled contained gametes. Non-reproductive colonies ranged in height up to 80 cm at the Outer Lagoon and 90 cm at the Rim Reef sites. Colonies up to 140 cm were non-reproductive at the Inner Lagoon, in association with the overall greater density of colonies at this reef zone. The presence of non-reproductive colonies above the height of first reproduction may be the effect of stress on the colony, for example, from previous resource allocation to repair from injury (Hall and Hughes, 1996; Kapela and Lasker, 1999).

4.5.3 Gamete development

Descriptive accounts of gorgonian reproductive cycles are aided by the relatively large size of gametes that enables observation by hand sections under a dissecting microscope. The gametes of *Pseudoplexaura porosa* are positioned as described for the other gorgonian species studied to date, at the base of the septa attached by a short mesogleal stalk (Bayer, 1974; Brazeau and Lasker, 1989; Brazeau and Lasker, 1990; Coma *et al.*, 1995a; Kapela and Lasker, 1999; Beiring and Lasker, 2000). The eggs and spermaries of *P. porosa* were both white, although in some species sex can be distinguished by gamete colour (Grigg, 1979; Brazeau and Lasker, 1989; Coma *et.al.*, 1995a). Many of the spermaries of *P. porosa* resembled the oocytes, as they remained circular through development. Sex determination of mature gametes was possible by the rigidity of the oocytes that held their shape after being handled, whereas the spermaries were easily deformed. Mature gametes filled the polyps of the colonies, a

mean of 1.04 oocytes >500μm polyp^{-1} and 12.32 spermaries polyp^{-1} maturing during each reproductive month. Gamete densities from the *P. porosa* colonies studied in Panama were expressed as cumulative totals over the three spawning months, female colonies with a mean of 4.33 oocytes polyp^{-1} and male colonies 64 spermaries polyp^{-1} (Kapela and Lasker, 1999). Estimating gamete densities per month from these values gives a comparable mean oocyte number (~1.44 oocytes polyp^{-1}), although spermary densities were greater in male colonies from the Panama population (~21 spermaries polyp^{-1}).

In all the gorgonian species studied to date, immature oocytes are found year round in female polyps and cohorts develop from this batch over each spawning month (Grigg, 1979; Brazeau and Lasker, 1989; Brazeau and Lasker, 1990; Coma *et al.*, 1995a; Kapela and Lasker, 1999; this study). In comparison, the presence of spermaries in male colonies is often seasonal. Spermaries were first discernible in the *P. porosa* colonies as swellings and 'grapes' four to five weeks before spawning. This is a result comparable to that of Kapela and Lasker (1999) in Panama where most of the spermatogenesis cycle was completed within a month. The spermary developmental times documented for other gorgonian species varied from three months for *Briareum asbestinum* (Brazeau and Lasker, 1990), six months for *Paramuricea clavata* (Coma *et al.*, 1995a) and small spermaries were present year round in *Muricea californica* and *M. fruticosa* with maturation over five months (Grigg, 1979). Extended periods of oogenesis in comparison to rapid spermatogenesis similarly occurs in some octocorals (Yamazato *et al.*, 1981; Benayahu and Loya, 1986) and also in most scleractinian species (see review by Harrison and Wallace, 1990). The developmental times represent the greater energy investment needed for the development of oocytes over spermaries (Charnov, 1982; Hall and Hughes, 1996).

4.5.4 Spawning

There was inter-annual variation in the timing of the reproductive season of the *Pseudoplexaura porosa* tagged colonies, and this is discussed in relation to the different annual temperature profiles described in Chapter 5. Spawning occurred in August, September and October in 1998 and was restricted to July and August in 1999 and 2000. Within each year, individual colonies spawned for either one or two months, and in 1998 three colonies spawned repetitively over three months. Inter-colony variability in the intensity and duration of reproductive effort within a population may be influenced by the unknown history of the colonies, such as previous injury that may cause a colony to cease reproduction (section 4.5.2; Hall and Hughes, 1996; Hall, 1997; Kapela and Lasker, 1999). Micro-environmental abiotic and biotic conditions may also be influencing colonies of the same population. A total of five tagged colonies (two colonies from the Inner Lagoon reef zone, one colony from the Outer Lagoon and two colonies from the Rim Reef) did not produce any mature gametes over the summer months of 2000. In contrast, all colonies were reproductively active in 1998 and 1999. Variation in coral fecundity throughout the lifetime of a colony may be part of a natural process, or is possibly the result of adverse environmental conditions in that year or the previous year diverting energy away from reproduction (Wallace, 1985; Rinkevich and Loya, 1987). Overall, the summer of 2000 was a cool summer and the lower temperatures may have been a contributing factor to the reduced reproductive effort (Chapter 5). Male colonies showed less inter-annual and inter-colony variability in fecundity compared to female colonies and only one of the five colonies that did not produce mature gametes in 2000 was male. Kapela and Lasker (1999) also found variability in fecundity greater among female colonies than males, presumably reflecting the lower energy required for the production of spermaries compared to oocytes (as discussed in section 4.5.3). Thus, female colonies are more susceptible to changes in colony condition or the effect of environmental factors.

The timing of spawning of *P. porosa* each month was similar to previous reports for this species in Panama (Lasker *et al.*, 1996; Coma and Lasker, 1997a; Kapela and Lasker, 1999). Spawning began six days after the full moon of July and August 1999 from Inner Lagoon colonies. The colonies kept in aquaria began spawning five days after the full moon, but as this was the day of collection, may be a stress response causing early

release of the mature gametes. Spawning occurred for 3-4 nights from colonies held in aquaria, continuing until 7-8 days after the full moon, and field collections 10 and 13 days after the full moon confirmed that spawning had ended from the previously gravid Inner Lagoon colonies. Gamete release commenced approximately 30 minutes after sunset and for the majority of colonies lasted for 30-40 minutes. The spawning of branches kept in aquaria occurred 10-20 minutes earlier than *in situ* colonies, which is in contrast to the spawning of *Plexaura kuna* branches in aquaria that lagged behind colonies in the field by 30-90 minutes (Brazeau and Lasker, 1989). Disruption of spawning times in aquaria is likely to be caused by external light pollution confusing the night irradiance cue of the lunar cycle. Some branches of *P. porosa* colonies released copious numbers of eggs in the field whilst adjacent branches released only a few, and this is a similar observation to the intra-colony variation in spawning intensity documented from colonies in Panama (Kapela and Lasker, 1999). Inter-colony variation in the timing and intensity of spawning has also been noted in colonies of *Plexaura kuna* (Brazeau and Lasker, 1989). Individual colony branches of *P. porosa* do not vary in gamete per polyp fecundity levels (Kapela and Lasker, 1999; section 4.4.6), suggesting that there is synchronous maturation followed by inter-branch variation in actual gamete release. This explains the extended spawning period of a few days for individual colonies (Brazeau and Lasker, 1989; Kapela and Lasker, 1999).

Lunar periodicity of spawning also occurs for the external brooding species *Briarieum asbestinum* (Brazeau and Lasker, 1990) and *Paramuricea clavata* (Coma *et al.*, 1995a), and for the broadcasting species *Plexaura kuna* (Brazeau and Lasker, 1989) and *Plexaura flexuosa* (Beiring and Lasker, 2000). In all species gamete release begins 3-6 days (dependent on the species) after the full moon lasting for a few days. There is some overlap in the day and time of spawning of *B. asbestinum*, *P. kuna* and *P. porosa* in Panama, and there has been *in situ* observation of multi-species egg release on the same nights 4-7 days after the full moon in July (Brazeau and Lasker, 1989; Lasker *et.al.*, 1996). Multi-species spawning of *Pseudoplexaura* sp., *Plexaura homomalla,* and *Pseudopterogorgia* sp. also co-occurred in Bonaire over a few nights 3-8 days after the full moon in August and September, coinciding with the broadcast spawning of several scleractinian species (de Graaf *et al.*, 1999). Mass spawning of alcyonaceans has been reported on the Great Barrier Reef, also occurring over the same days as the scleractinian coral spawning (Alino and Coll, 1989). The synchronous spawning of

gonochoric species on specific days is essential to ensure gamete mixing and fertilisation. Multi-species mass spawning events are a likely consequence of timing to the same proximate cue of the lunar cycle, although may also benefit propagule survival by predator satiation (Harrison *et al.*, 1984; Alino and Coll, 1989). The release of gametes after the full moon spring tides when tidal amplitude is lower is presumed to reduce gamete dilution (Babcock *et al.*, 1986) and thereby enhance fertilisation rates that are strongly disrupted by currents (Lasker *et al.*, 1996; Coma and Lasker, 1997a; Coma and Lasker, 1997b).

4.5.5 Fecundity and polyp volume variation

Pseudoplexaura porosa colonies from the replicate Inner Lagoon sites had a significantly greater mean polyp volume than colonies from Crescent C at the Outer Lagoon and colonies from Hog Breaker at the Rim Reef. All tagged colonies were selected to be of approximately the same height (80-100 cm tall) and so polyp volume variation as a function of colony size was not investigated. However, the larger polyps of the colonies at the Inner Lagoon are consistent with the abundance of taller colonies at this reef zone, in comparison to the more branched and 'bushier' colonies offshore (section 4.4.2 and 4.5.2). Thus, the results suggest that some colonies exhibiting a slender form by diverting growth away from lateral branching also extend individual polyp growth. The lateral growth of a bushier colony is at the expense of upward colony extension as well as causing a reduction of polyp growth. This is not a significant trend for all of the sites indicating intra-zone as well as inter-colony variation to colony and polyp growth.

There is a positive relationship between polyp volume and gamete volume per polyp between *P. porosa* colonies. Previous studies relating coral fecundity have largely been concerned with the relationship between increasing colony height for gorgonian species (Brazeau and Lasker, 1990; Coma *et al.*, 1995b; Beiring and Lasker, 2000), or surface area for scleractinian species (Rinkevich and Loya, 1979a; Kojis and Quinn, 1981b; Richmond, 1987; Babcock, 1988). Van Veghel and Kahmann (1994) and Sakai (1998) did address the issue of polyp size affecting fecundity and found that the percentage of polyps with gametes was greater with an increased polyp size in *Montastrea annularis*

and *Goniastrea aspera* respectively. Fecundity was also correlated with increasing polyp size in the deep-sea solitary coral *Fungiacyathus marenzelleri* (Waller *et al.*, 2002). Harriott (1983a) similarly suggested that polyp size limits gonad production in *Lobophyllia corymbosa*, although gave no quantitative data. Shlesinger *et. al.* (1998) found that fecundity (eggs per polyp) increased with polyp size between different species but did not study the relationship within a species. The positive relationship between polyp size and fecundity is indicative, as mechanically there is more space available for gametes in larger polyps, although the relationship will be dependent on the energy budget available to growth and reproduction. However, addressing polyp size as well as colony size is important when considering colony fecundity.

4.5.6 Intra-branch variation in oocyte fecundity

There was no variation in oocyte fecundity discernible between the two branch distances of 3-5 cm and 8-10 cm from the branch tip of the *P. porosa* colonies. The location of mature polyps at just 3 cm down the branches of *Pseudoplexaura porosa* indicates fast growth to maturity of polyps located at the new branch tips. Immature portions of colonies that are still actively growing will have less energy available for reproduction, and a decrease in reproductive activity in such areas has been shown in other gorgonian species (*Plaxaura kuna*, Brazeau and Lasker, 1989; *Briareum asbestinum*, Brazeau and Lasker, 1990; *Paramuricea clavata*, Coma *et al.*, 1995b), as well as in some branching scleractinian species (*Stylophora pistillata*, Rinkevich and Loya, 1979a; and *Acropora* spp., Wallace, 1985). Gamete number was greatest in the central regions of *Briareum asbestinum* and as well as decreasing within 5 cm of the tip, was also lower at the base of the branch (Brazeau and Lasker, 1990). Any variation in oocyte fecundity below 8-10 cm from the *P. porosa* branches was not investigated and so it is not known whether central branches are more fecund. Kapela and Lasker (1999) found no difference in fecundity between peripheral and central branches of *P. porosa* colonies in Panama.

4.6 Summary

Spicule analysis of *P. porosa* colonies in Bermuda confirms a high variability of spicule types both within and between the colonies. The original descriptive work of Bayer (1961) is not accurate for the Bermuda population and the presence of 'spiny spindles' (gradations between the clubs and the unilateral spiny spindle) replaces the 'unilateral spiny spindle' as a diagnostic feature for *P. porosa* in Bermuda. The abundance of *Pseudoplexaura porosa* colonies across the Bermuda platform is greatest at the Inner Lagoon and Rim Reef zones. The lower density of colonies at the Outer Lagoon may indicate a patchy distribution or may be particular to the area studied, and a further survey is needed to clarify this. The overall sex ratio of the gonochoric colonies was not significantly different from unity, although there was a slight bias towards both male and female colonies at the different reef zones, possibly reflecting vegetative proliferation in some areas or just an insufficient sample size.

The reproductive season for *P. porosa* in Bermuda was over the summer months of July and August, with spawning extending into September and October after the warm summer of 1998. There was inter-colony variability in the intensity and duration of spawning both within and between years. Variation in colony fecundity may be a natural process, the result of adverse environmental conditions or associated with the history of the colonies, such as previous injury and resource allocation to repair. *P. porosa* spawning occurred on a similar lunar cycle as colonies in the Caribbean, beginning six to seven days after the full moon and lasting for three to four days. Gamete release commenced approximately 30 minutes after sunset and lasted for 30-40 minutes. The *P. porosa* colonies growing in the inshore environment of the Inner Lagoon were generally taller, more slender and had a greater polyp volume that the smaller, bushier colonies growing offshore. There was a positive relationship between the increased polyp volume of the inshore colonies and gamete volume per polyp. Oocyte fecundity did not vary within a colony with distance from the branch tip, although there was intra-colony variation in the day of actual release of mature gametes leading to the extended spawning periods of a few days for individual colonies.

Chapter 5: Contrasting effects of temperature on the reproduction of a brooding scleractinian and a broadcasting gorgonian from sub-tropical Bermuda

5.1 Introduction

The role of seawater temperature in controlling the reproductive cycles of various coral species has been well documented (Orton, 1920; Kinne, 1963; Giese, 1987; Harrison and Wallace, 1990; see Chapter 1). Experimental studies directly exposing corals to a range of temperatures have shown a narrow threshold for reproductive success that is species and location specific (Jokiel and Guinther, 1978; Jokiel *et al.*, 1985; Ward *et al.*, 2000). Changes in seawater temperatures on a global scale have recently been reported, and it is clear that thermal cycles are not stable (Houghton *et al.*, 1996). Many coral populations are surviving at their thermal limits of successful growth and reproduction, and small increases in temperature have caused a decline in coral health as a result of both bleaching and depressed growth (Brown and Odgen, 1993; Hoegh-Guldberg, 1999; Souter and Linden, 2000). Reproduction is the process that leads to recovery from disturbance and the population of new areas, and is critical for the survival of coral reefs. It is therefore essential to understand the ways in which temperature controls this sensitive part of the coral life cycle so that any long term effects of changing temperature profiles on coral populations can be assessed.

Studies across the Pacific have shown that, when other environmental parameters appear stable, the timing of the reproductive season corresponds with the favourable temperature period for each given species, leading to a latitudinal variation that is dependent on the timing of the annual temperature maximum (Kojis and Quinn, 1981a; Kojis and Quinn, 1981b; Stoddart, 1985; Kojis, 1986b; Yamazato *et al.*, 1991; Dai *et al.*, 1992; Hayashibara *et al.*, 1993; review in Chapter 1). Homogeneity of the timing and duration of planula release or spawning occurs in several scleractinian species studied from the central and lower Caribbean (Szmant, 1986, 1991; Soong, 1991; Gittings *et al.*, 1992; Steiner, 1995). Sea surface temperatures at lower latitudes within the Caribbean closely mimic the stable air temperature regimes in this tropical and

predominantly maritime region (CARICOMP, 1997). Except in areas of local upwelling, similar oceanographic conditions mitigate both the annual and diurnal seawater temperature range (Steiner, 1995). Uniformity of reproductive characters is also suggested to be a consequence of high gene flow in the area (Soong, 1991). However, with the increasing seasonality at greater latitudes, uniformity of breeding seasons may not extend to the Northern Caribbean and Bermuda, where many coral species are at their distribution extreme (Chapter 2).

The study of the brooding Scleractinian, *Porites astreoides,* outside the central Caribbean showed a shorter reproductive season at the North Florida Keys related to the greater temperature range at the higher latitude (McGuire, 1998). The present study further investigates latitudinal variation in the breeding season of *P. astreoides* in Bermuda, the northern distribution extreme of this species. In addition, the reproductive season for the broadcasting gorgonian species, *Pseudoplexaura porosa,* in Bermuda is compared to the information available for this species at the southern Caribbean reefs of Panama (Lasker *et al.*, 1996; Coma and Lasker, 1997a; Kapela and Lasker, 1999). The timing of the breeding season at the different latitudes is discussed in relation to the controlling effect of the annual seawater temperature patterns on gamete and planulae maturation.

Aspects of the reproductive biology of *Porites astreoides* in Bermuda are presented in Chapter 3 and the reproductive cycle and features of the population biology of *Pseudoplexaura porosa* in Bermuda presented in Chapter 4. This chapter focuses on temperature as the factor controlling the breeding season and reproductive effort of these species. The effects of both inter-annual temperature variation on the reproductive cycles, as well as the different temperature profiles of the three physiographic reef zones within the 18 km wide Bermuda platform, are investigated (see Chapter 2 for a description of the Bermuda platform and temperature profiles). The pronounced temperature gradient across the reef zones of Bermuda provide an ideal opportunity to study the effect of variable seawater temperature profiles on the reproductive effort of conspecifics occurring at different sites within a defined region.

The study species differ in their taxonomic status (Scleractinia versus Gorgonacea) and also in their reproductive mode (brooding versus broadcasting). Sexuality is also

inconsistent between the species, as *Pseudoplexaura porosa* is gonochoric (Chapter 4) whereas *Porites astreoides* demonstrates a mixed sexuality of both gonochorism and hermaphroditism (Chapter 3). Coral species that brood their planulae internally generally reproduce over an extended breeding season compared to broadcast spawning species, inferring differential energy allocation to each reproductive mode (Fadlallah, 1983a; Harrison and Wallace, 1990; Richmond, 1997). Thus, the effects of environmental changes on the timing of the breeding season and on reproductive effort are likely to differ between reproductive modes. Although the environmental factors controlling reproduction in scleractinian species have been well studied (Chapter 1), similar research on gorgonians is scarce (Chapter 4). There is limited information on the differences in the reproductive cycle of the same species from different geographic locations or on populations occurring under stressful conditions. Gorgonian species may respond to biotic and abiotic changes in the same manner as physiologically similar scleractinian species, but the knowledge of gorgonian reproductive cycles is too limited to make such assumptions. Indeed, several aspects of life history traits have already been shown to differ between the sub-classes. Gorgonian corals differ from scleractinian species in that their sexuality is dominated by gonochorism and they include surface brooding as a reproductive mode (Chapter 1). Juvenile gorgonians exhibit differences in their allocation of energy to growth and reproduction, with the majority of species delaying sexual maturity to a greater age than scleractinian species (Chapter 1). Information on the timing of the breeding season and reproductive effort of these coral species in Bermuda will provide insights into the limiting effect of temperature on coral reproduction, and further investigate the differences and similarities of the reproductive biology of closely related sub-classes of corals and different reproductive modes.

5.2 Objectives

This study addresses the following questions:

1. Does the timing and duration of the reproductive seasons of *Porites astreoides* and *Pseudoplexaura porosa* differ between Bermuda and the Caribbean?
2. Is there a variation in the reproductive effort of colonies of each species from the different reef zones of the Bermuda North Lagoon?
3. Is there inter-annual variation in the reproductive effort of the two species?
4. Can a relationship be defined between the reproductive effort of *Po. astreoides* and *Ps. porosa* colonies and seawater temperature profiles?

5.3 Methods

5.3.1. Seawater temperature data

Seawater temperature was monitored *in situ* using calibrated Onset Stowaway data loggers (accuracy ±0.05 °C, resolution ±0.02 °C) placed at each replicate site in the three physiographic reef zones of the Bermuda North Lagoon from January 1998 to December 2000 (see section 2.4, Chapter 2). Identical data loggers were used to record the seawater temperature in the aquaria where the *Porites astreoides* colonies were held to monitor larval release over the new moon periods of July, August and September in 2000.

5.3.2 Data collection

Porites astreoides planula release

Reproductive effort of colonies of the brooding species, *Porites astreoides*, was measured by the number of planula released. The seasonal release of planulae from *Porites astreoides* colonies in Bermuda occurs in July and August with a small number of planula released in September (Chapter 3). Planula release occurs over an approximately 20 day period, with a weak peak over the new moon. Colonies were therefore collected from reef sites at the Inner Lagoon, Outer Lagoon and Rim Reef (Figure 2.1, Chapter 2) between 7 and 10 days before each new moon (subject to weather conditions) of July, August and September in 1999 and 2000, and monitored in aquaria for 14-21 days (for sampling schedule see Table 3.1, Chapter 3). The colonies collected from all reef zones were monitored for the same time period each month. In 1999, the sample size was five colonies from each of the three reef zones in July and September, and was increased during August to ten colonies from the Inner Lagoon and eight colonies from the Outer Lagoon and Rim Reef. In 2000, the sample size in each month was ten colonies from each reef zone, with five colonies collected from two replicate reef sites within each reef zone.

All colonies collected were approximately 15 cm in diameter, were of the green colour morph and had no mucus covering. The entire colony was removed from the substratum using a hammer and chisel. All corals were transported back to the laboratory in coolers of seawater kept at ambient temperature to minimise collection stress. The corals were maintained on the shaded outdoor wet bench facility at BBSR. Each colony was placed inside a 'planulae collector', which was a clear Tupperware container (2.5 litre; 18 cm x 12 cm deep) with a water flow inlet at the base to promote circulation. The planulae collectors were placed within large aquaria of flowing seawater to help maintain a constant temperature. The container lids had a large window cut out which was covered in 200μm nitex mesh to allow water to flow out whilst retaining planulae within the container. The planulae of *Po. astreoides* are released after sunset (McGuire, 1998; pers. obsv.) and so the collectors were checked each morning. All planulae were removed using a pipette and counted. The lids to the

collectors were left off during the day to encourage water flow around the colonies and reduce shading. At the end of the monitoring period the surface area of the colonies was measured using the tin foil technique (Marsh, 1970). The colonies were then returned to their respective reefs and cemented in place. New colonies were collected over each new moon period to minimise the potential confounding effects of stress that may result from the corals being held in aquaria over extended periods. Reproductive effort for each colony was recorded as the total number of planula released per cm^2. The variable periods that colonies were monitored in aquaria each month (Table 3.1, Chapter 3) was accounted for by adjusting the measure of reproductive effort to the total number of planulae released per cm^2 per day. Mean reproductive effort was calculated for each reef site, zone and month.

Pseudoplexaura porosa gamete maturation

Reproductive effort by the broadcasting species, *Pseudoplexaura porosa*, was measured by determining the oocyte or spermary volume per polyp of the gonochoric colonies just before spawning. Mature gametes are present in *Pseudoplexaura porosa* adult colonies in Bermuda over the summer months of July/August until September/October (Chapter 4). The timing of spawning is on a similar lunar cycle to the Caribbean (Lasker *et al.*, 1996a; Kapela and Lasker, 1999), and begins 6-7 days after the full moon and continues for 3-4 days (Chapter 4). In 1998, five mature *P. porosa* colonies were tagged at Crescent C of the Outer Lagoon and Hog Breaker of the Rim Reef (Figure 2.1, Chapter 2). In 1999 and 2000 the sampling regime was increased to include replicate study sites at each of the three reef zones: Inner Lagoon, Outer Lagoon and Rim Reef. The sample size at each site included those colonies monitored in 1998 with a number of additional colonies varying between five and eight per site (Appendix 4.1C). The same colonies were repeatedly sampled each month and year to monitor gamete development within individual colonies. All colonies were sampled just before spawning in July-October 1998-2000 (see Appendix 4.1A and B for sampling schedule).

Sampling was carried out by SCUBA divers using pliers to collect two branch tips approximately 15 cm long from each colony. The samples were fixed in 9% seawater formalin for 36hr and then transferred to 70% EtOH before examination. Cross sections

along the branches were made by a sharp razor blade and whole polyps dissected out and observed using a dissecting microscope at x25 and x50 magnification. If the branch was immature, the other branch collected from each colony was checked for gamete presence to allow for intra-colony variability in maturation. Only one extra branch was collected to limit stress to the colonies. All spermaries were counted and measured within 10 polyps per branch at 8-10 cm from the branch tip of the male colonies. The oocytes in female colonies were less abundant and so a total of 20 polyps per branch were examined. The gametes were measured and placed into size classes with 100μm divisions ranging from <100μm to 700-800 μm. Total oocyte or spermary volume per polyp for each colony was then calculated assuming a sphere of the maximum diameter, d, per size class and using the formula $^4/_3\pi(d/2)^3$. Some spermaries develop slightly oval in shape when mature but were classified as spherical for the purpose of this study. The total spermary volume per polyp for each spawning month represents male reproductive effort as all the spermaries are shed during spawning. Oocyte volume was further grouped into mature (>500μm), which are those oocytes that are spawned, and immature volume (<100-499μm), which are present in the polyps outside of reproductive months. The calculated total oocyte or spermary volume per polyp for each colony is normalised against the mean polyp volume of that colony, which was shown to vary among the reef zones (Figure 4.12, Chapter 4).

5.3.3 Statistical Analysis

Transformation of the data

The *Porites astreoides* planula release data and the *Pseudoplexaura porosa* oocyte and spermary volume data were tested for conformity to the parametric assumptions of homogeneity of variances and normality, using the Bartlett and Kolmogorov-Smirnov tests respectively (Sokal and Rohlf, 1995). Arc sine transformation was necessary to remove variance heterogeneity among the *Porites astreoides* samples (Appendix 5.1) and the *Pseudoplexaura porosa* samples (Appendix 5.2). After transformation, two of the 15 samples from the *Po. astreoides* data were significantly different from a normal distribution (Appendix 5.3). Caution must therefore be taken with the use of parametric

statistics on these data, although ANOVA is known to be fairly robust to non-normality (Sokal and Rohlf, 1995). The *Ps. porosa* oocyte and spermary volume data were normally distributed after arc sine transformation (Appendix 5.3).

ANOVA design

The *Porites astreoides* and *Pseudoplexaura porosa* data were separately analysed, each using a nested ANOVA design to examine for the variance between the zone in which the samples were collected, with the replicate reef sites nested within each zone, and the month of collection. The format in which the temporal factor of month was incorporated into the ANOVA was determined by the sampling method employed, as this effected the independence of the data (Underwood, 1997). Separate *Po. astreoides* colonies were sampled from the study populations in each month, ensuring independence of the data and allowing month to be a level of nesting in the ANOVA design (performed using BIOMstat version 3.0). The data across 1999 and 2000 were therefore analysed using a two-level nested ANOVA to compare the variance among the months, with zone nested within months. The ANOVA was followed by multiple comparisons of means using the Tukey-Kramer and GT2 method as defined by Sokal and Rohlf (1995). In 2000, samples were consistently collected from replicate reef sites in each reef zone over all months to examine for intra-zone variation in the reproductive effort of the study species. A three-level nested ANOVA was therefore run on the *Porites astreoides* data from 2000 to compare the variance among the reef sites nested within zone and within month. Testing for intra-zone variation was performed prior to grouping the reef sites within the zones for the ANOVA analysis across both years.

In contrast, the *Ps. porosa* data are not independent as the same colonies from each population were repeatedly sampled each month in order to monitor gamete maturation from individual colonies. The time factor is therefore incorporated into the ANOVA design as a repeated measure, instead of a level of nesting (Wilkinson *et al.*, 1992) to compare the variance among the months, with zone further analysed within months. The oocyte and spermary data from the gonochoric *Ps. porosa* colonies were analysed separately, each combined over the years. The data from 1998 could not be included as the Inner Lagoon was not sampled in this preliminary study and only one site per zone

was monitored. Intra-zone variation in *Ps. porosa* oocyte and spermary reproductive effort was incorporated into each ANOVA across both years, the replicate reefs forming one level of nesting within the zones, with month as a repeated measure (performed using SYSTAT Version 5.2). Multiple comparisons of means were performed for both the oocyte and spermary ANOVAs using the Tukey-Kramer and GT2 method as defined by Sokal and Rohlf (1995).

5.4 Results

5.4.1 Seawater temperature data

The annual seawater temperatures cycles at the Inner Lagoon, Outer Lagoon and Rim Reef zones of the Bermuda North Lagoon from January 1998 until December 2000 were presented in section 2.4, Chapter 2. The seawater temperature of the aquaria holding the *Porites astreoides* colonies was monitored over the new moons of July to September in 2000. The aquarium seawater followed a similar temperature profile to the reef zones, predominantly remaining intermediate to the Rim Reef and the Outer Lagoon and never exceeding the temperature of the Inner Lagoon (Figure 5.1). The range of temperatures in the aquaria was greater than that of the reef zones (Figure 5.2). The small body of water in the aquaria is vulnerable to heating and cooling by the fluctuating air temperature. The mean daily maximum temperature in the aquaria does not increase above that of the reef zones as maximum air temperatures in the summer are close to that of the seawater and therefore the corals were not stressed by abnormally high temperatures. However, air temperatures at night fell to a mean minimum of 23.6°C in July, 24.1°C in August and 23.7°C in September (Bermuda weather service, Internet report), which was below the average seawater temperature for those months. The relatively weak flow into the aquaria was not sufficient to maintain the temperature as that of the seawater feeding the system. The falling air temperature at night cooled the aquarium seawater to 1.6-2.5°C lower than the maximum mean daily values. In comparison, the diurnal range of the seawater temperature at the reef zones

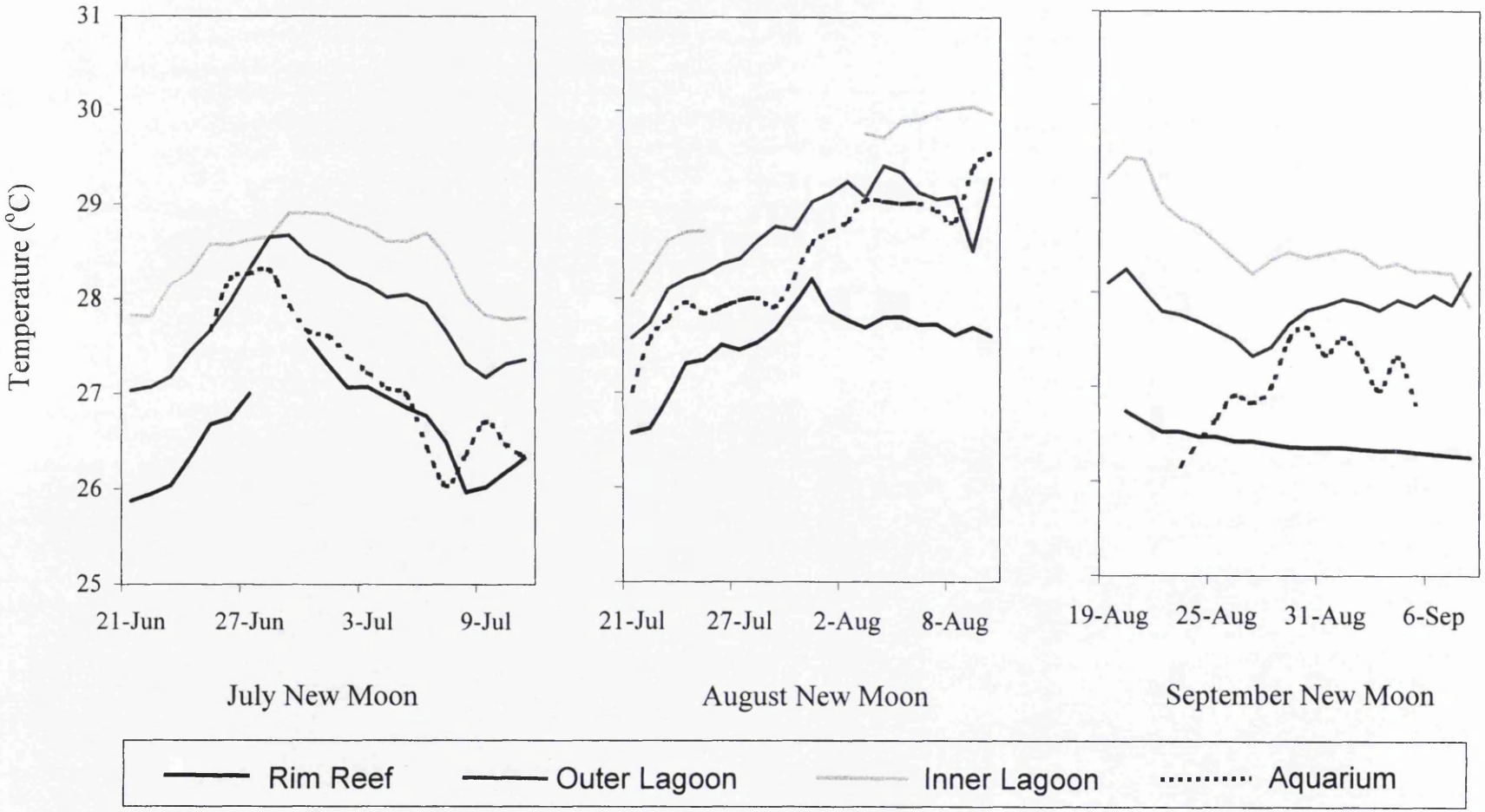


Figure 5.1: Aquarium and reef seawater temperature data for 10 days either side of the new moon in the summer months of 2000. Lines represent mean daily values. Gaps in the data are when the temperature loggers were not deployed, or malfunctioned.

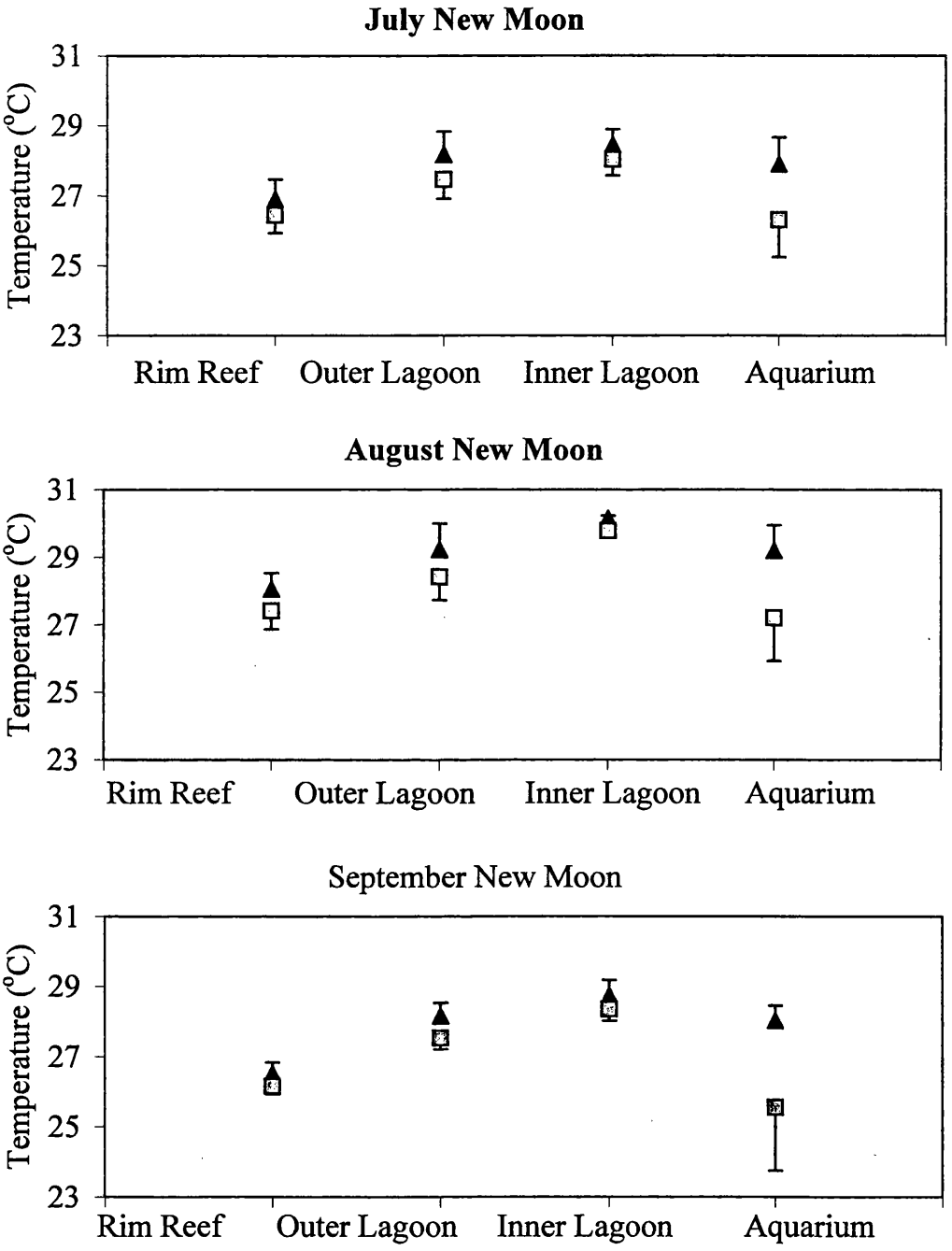


Figure 5.2: Mean daily maximum (triangle) and minimum (square) seawater temperatures at the three reef zones and in the aquaria for 10 days either side of the new moon in the summer months of 2000. Error bars are +1SD for maximum values and -1SD for minimum values.

was only 0.4-0.7°C. However, the minimum temperatures experienced by the corals kept in the aquaria were still within their natural annual threshold.

5.4.2 *Porites astreoides* planula release

Variation in reproductive effort

The overall reproductive effort from the *Porites astreoides* colonies (mean number of planulae per cm^2 per day) varied with the month (July to September) and the reef zone (Inner Lagoon, Outer Lagoon and Rim Reef) in 1999 and 2000, both in terms of the numbers of planulae released (Figure 5.3A) and the percentage of colonies releasing planulae (Figure 5.3B; see Appendix 5.5 for raw data). Over the summer of 1999, the greatest monthly release of planulae from the Inner Lagoon and Outer Lagoon colonies occurred in July, reproductive effort decreasing in August, and no planulae were released in September 1999 (Figure 5.3A). In contrast, reproductive effort from the Rim Reef colonies was greatest in August 1999 and planulation continued into September (Figure 5.3A). With the exception of the Outer Lagoon, the percentage of colonies planulating each month declined over the summer in 1999 (Figure 5.3B). The percentage of reproductively active colonies at the Outer Lagoon increased from July to August 1999 (Figure 5.3B) even though overall reproductive effort from the colonies decreased (Figure 5.3A).

In the following year, reproductive effort of the colonies from the Outer Lagoon, as well as the Rim Reef was greatest in August, and only the Inner Lagoon colonies followed the trend seen in 1999 and released more planulae in July than in August (Figure 5.3A). In contrast to the small increase in 1999, the number of planula released from the Rim Reef colonies between July and August 2000 increased more than four times (Figure 5.3A). The percentage of colonies planulating from the Rim Reef and Inner Lagoon zones was greatest in August 2000 (Figure 5.3B). There was a decrease in the number of reproductive colonies from the Outer Lagoon between July and August 2000, although only by one colony. There was a small reproductive effort from colonies at the Outer Lagoon and Rim Reef zones in September 2000 (Figure 5.3A), when a low percentage of colonies released planulae (Figure 5.3B).

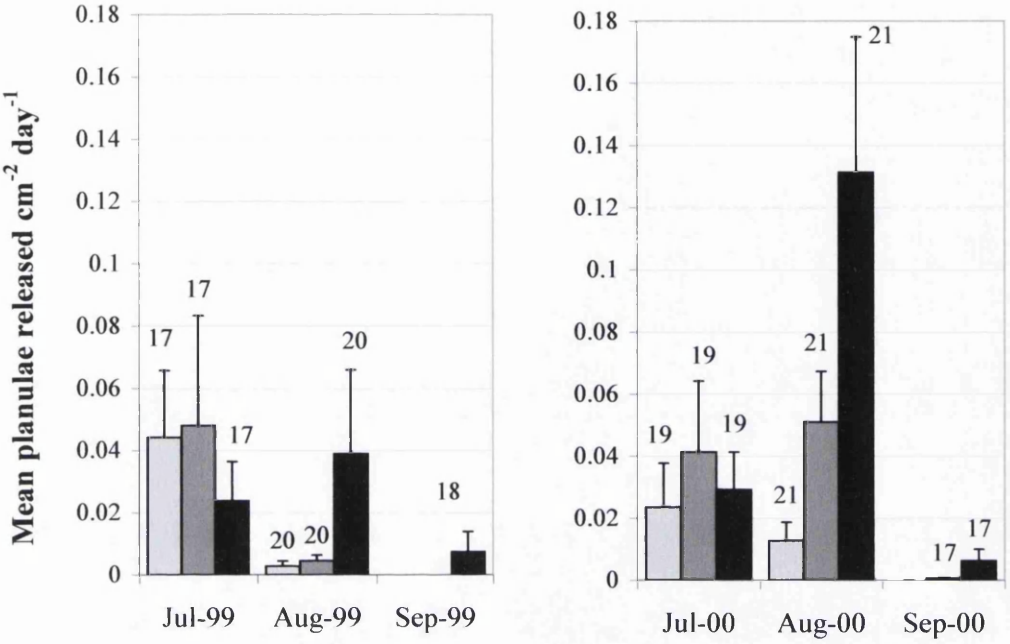


Figure 5.3A: Mean number of *Porites astreoides* planulae released from each reef zone over the summer months of 1999 and 2000. Figures were normalized to the number of days the colonies were kept in aquaria (shown above the bars). Error bars are +1 standard error. See Figure 19B below for the number of colonies sampled.

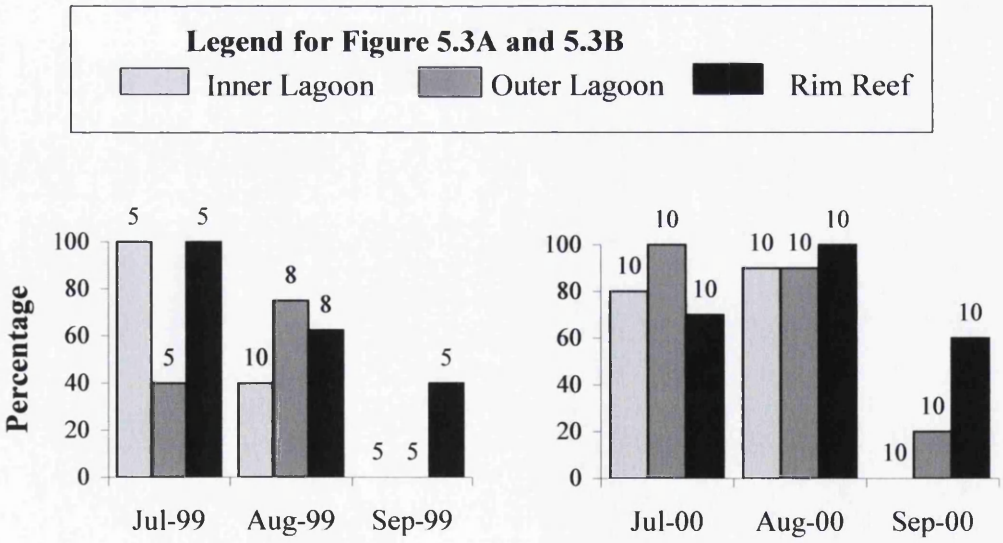


Figure 5.3B: The percentage of *Porites astreoides* colonies that released planulae over the summer months of 1999 and 2000. The number of colonies sampled is shown above the bars.

Table 5.1: One-way ANOVA with three levels of nesting to examine for intra-zone variation in the reproductive effort of *Porites astreoides* colonies (arc sine transformed) from the replicate reef sites nested within each zone (Inner Lagoon, Outer Lagoon and Rim Reef) and within the months over the summer of 2000. See section 5.3.3 for details of the ANOVA design.

Level	SS	df	MS	Fs	P
Reef within zone	3.605	9	0.401	0.961	0.528
Reef within month	12.040	15	0.802	1.925	0.395
Error	30.02	72	0.417		

There was no significant intra-zone variation in the reproductive effort of the *Po. astreoides* colonies monitored at the replicate reef sites in 2000 (Reef within zone, P= 0.528, Table 5.1), or between the replicate reef sites and the months (Reefs within month, P= 0.395). Therefore, the reproductive effort of the colonies from the replicate reef sites in each of the three zones was combined to give *Po. astreoides* reproductive effort per reef zone in 2000.

Table 5.2: One-way ANOVA with two levels of nesting to examine for differences between the reproductive effort of *Porites astreoides* colonies (arc sine transformed) in each month over 1999 and 2000, with zone (Inner lagoon, Outer Lagoon and Rim Reef) nested within months. See section 5.3.3 for details of the ANOVA design.

Level	SS	df	MS	Fs	P
Month	22.966	5	4.593	5.913	0.006
Zone	32.287	17	1.899	5.425	$5.792\ 10^{-9}$
Zone within month	9.322	12	0.777	2.219	0.195
Error	44.809	128	0.350		

The combined reproductive effort (mean number of planulae per cm^2 per day) of the *P. astreoides* colonies across all the months in 1999 and 2000 was significantly different between the reef zones (Zone, P= $5.792\ 10^{-9}$, Table 5.2). The post-hoc test revealed that the large overall reproductive effort from the Rim Reef colonies, caused by the high number of planulae released in August 2000, was significantly different to that of the weak combined reproductive effort from the Inner Lagoon colonies (Appendix 5.7).

There was also a significant difference between *Po. astreoides* reproductive effort over the months, after combining the planula release data from colonies at all the reef zones (Month, P= 0.006, Table 5.2). The large reproductive effort in August 2000 significantly differed from September 2000 and August and September 1999 (post-hoc test; Appendix 5.7). In addition, the low reproductive effort from September in 1999 and 2000 were each significantly different to that of July 1999 and July and August 2000 (Appendix 5.7). However, this trend of a greater reproductive effort from the *Po. astreoides* colonies later in the season at the Rim Reef compared to colonies from the other reef zones was not strong enough to show a significant difference between the individual zones over the months (Zone within month, P= 0.195, Table 5.2).

The effect of temperature

Variation in monthly and annual seawater temperature in Bermuda was investigated as a possible causal factor for the observed differences in the reproductive effort from colonies collected from the reef zones, and over different time scales (months to years). The seawater temperature profiles over the reef zones in Bermuda are presented in section 2.4, Chapter 2, and are shown in Figure 5.8 in relation to *Pseudoplexaura porosa* spawning. Planula development through brooding is assumed to take from 2-3 weeks (McGuire, pers. comm), and so the average seawater temperature for the 30 days preceding the new moon at each reef zone was related to the combined reproductive effort for that month. There was a negative relationship between the mean number of planulae released (same data as Figure 5.3A) and the average seawater temperature for the preceding lunar cycle for each month (Figure 5.4A). This relationship was significant at the 5% probability level (Product-moment correlation coefficient on arc sine transformed data; P= 0.003, r= -0.672, n =17). Planula release occurred when temperatures for the month preceding the new moon reached above 26.5°C, except for the Rim Reef zone in July 2000, when planula release occurred even though the preceding average temperature was 24.9°C. In this comparatively cool summer, reproductive effort was lower in July compared to the large number of planula released in August 2000 (Figure 5.3A), when temperatures at the Rim Reef were above the

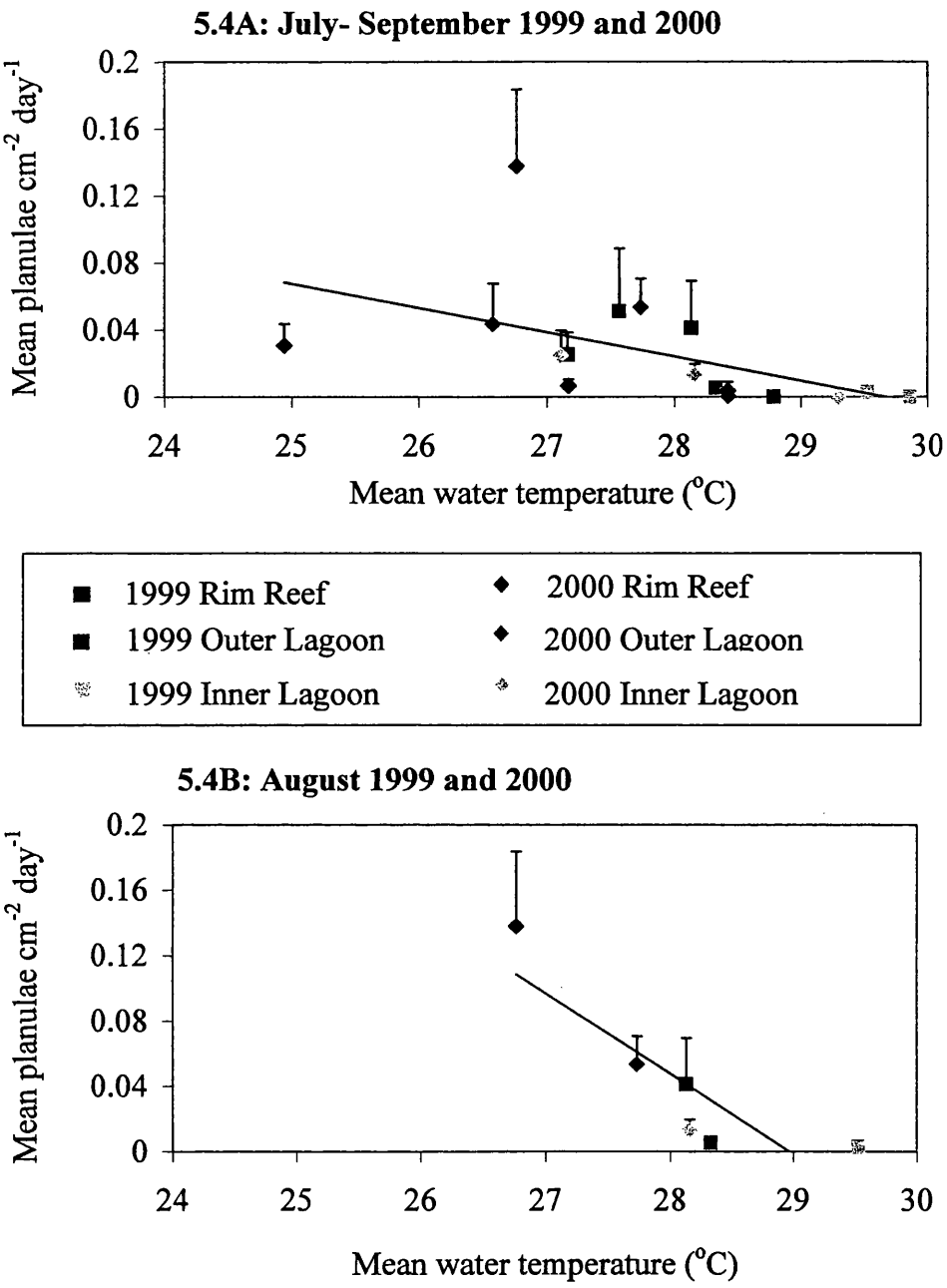


Figure 5.4: The relationship between seawater temperature and the number of planula released (+1SE) by *Porites astreoides* colonies for (A) July to September 1999 and 2000 and (B) August only 1999 and 2000. Water temperature is the average for the lunar cycle (30 days) preceding the new moon for each month. Trendlines are linear regressions; see text for correlation analysis on transformed data. No temperature data are available for the Inner Lagoon in July 1999.

optimum at 26.8°C (Figure 5.4A). When seawater temperatures reached in excess of 28.5°C, as occurred prior to the September new moon at the Inner Lagoon and Outer Lagoon in 1999 and the Inner Lagoon in 2000, planula release ceased from the monitored colonies. A small reproductive effort was recorded from the Rim Reef colonies in September 1999 and the Outer Lagoon and Rim Reef colonies in September 2000 (Figure 5.3A) as temperatures remained below 28.5°C. An exception to this trend of higher temperatures causing the cessation of planula release was a small release of planulae from the Inner Lagoon in August 1999, when the average temperature for the preceding lunar cycle reached 29.5°C.

The inter-annual variation in reproductive effort between 1999 and 2000 was greatest in the month of August (Figure 5.3A). The data from each year in this month alone was also plotted against the average seawater temperature for the preceding lunar cycle, and clearly demonstrated the negative relationship depicted by both inter-zone and inter-annual variation in planula release (Figure 5.4B; Product-moment correlation coefficient on arc sine transformed data; $P= 0.014$, $r= -0.903$, $n =6$). The monitored corals from each reef zone produced more planulae in August 2000, when seawater temperature in that zone was lower than in August 1999. Within each year, reproductive effort was greatest from the Rim Reef colonies when the average seawater temperature was lower, and the gradient of increasing temperature across the reef zones to the Inner Lagoon corresponded to a decline in reproductive effort.

5.4.3 *Pseudoplexaura porosa* gamete maturation

Variation in reproductive effort

Mature oocytes were first present in the *Pseudoplexaura porosa* colonies in August 1998 (Figure 5.5; see Appendix 5.6 for all raw data). In July 1998, only immature oocytes (<500μm) were observed, and these are present year round in mature female colonies (Chapter 4). The male colonies were full of numerous small spermaries (<200μm) in July 1998, comprising a weak reproductive effort (Figure 5.6). However, it is presumed that this was the immature cohort growing for spawning in August 1998

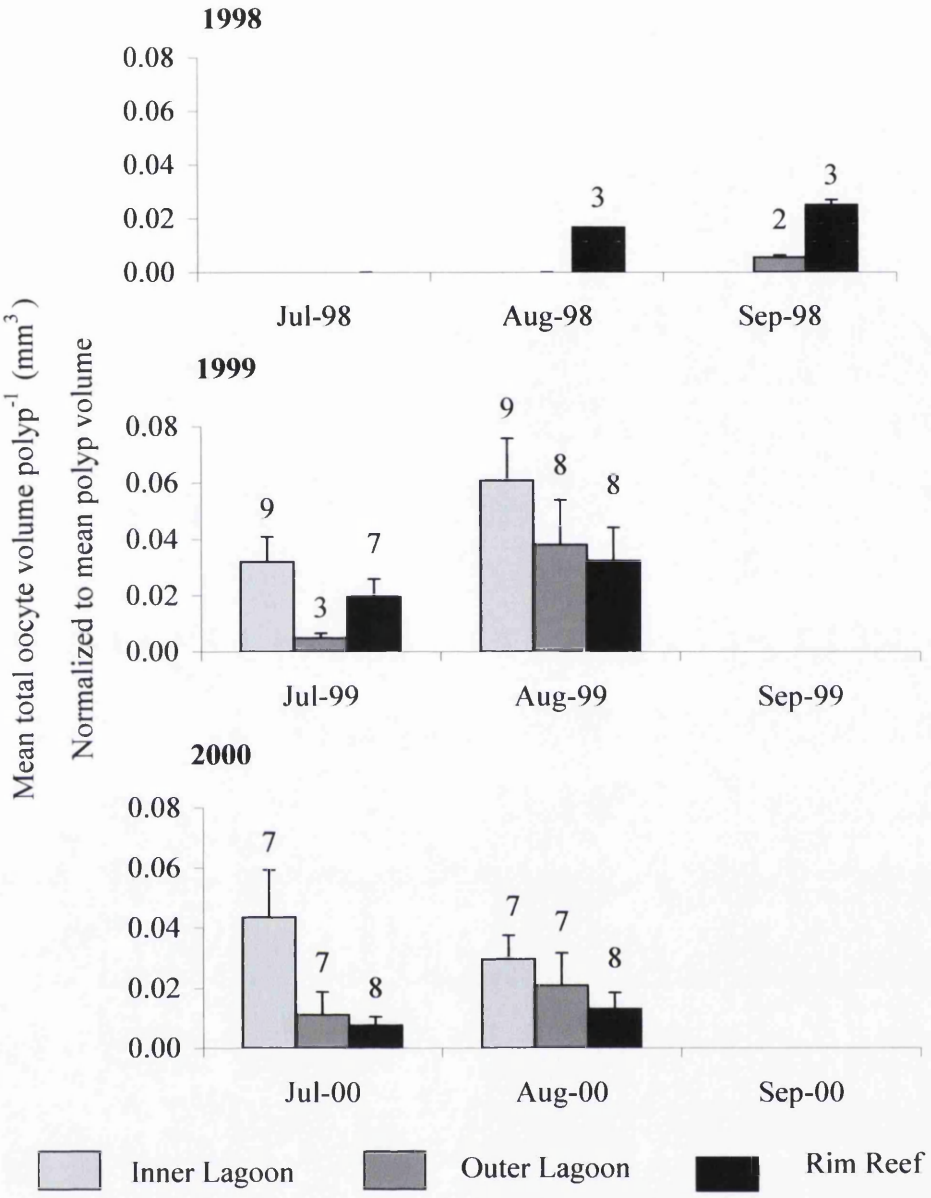


Figure 5.5: *Pseudoplexaura porosa* oocyte reproductive effort combined from all colonies for each reef zone. Shown for the reproductive months of July-September 1998-2000. Mature oocytes were also present in October 1998 from the Rim Reef with a mean total oocyte volume of 0.004 mm^3 $polyp^{-1}$ (n=3). The number of colonies the mean is calculated from is shown above the bar. Error bars are +1 Standard Error.

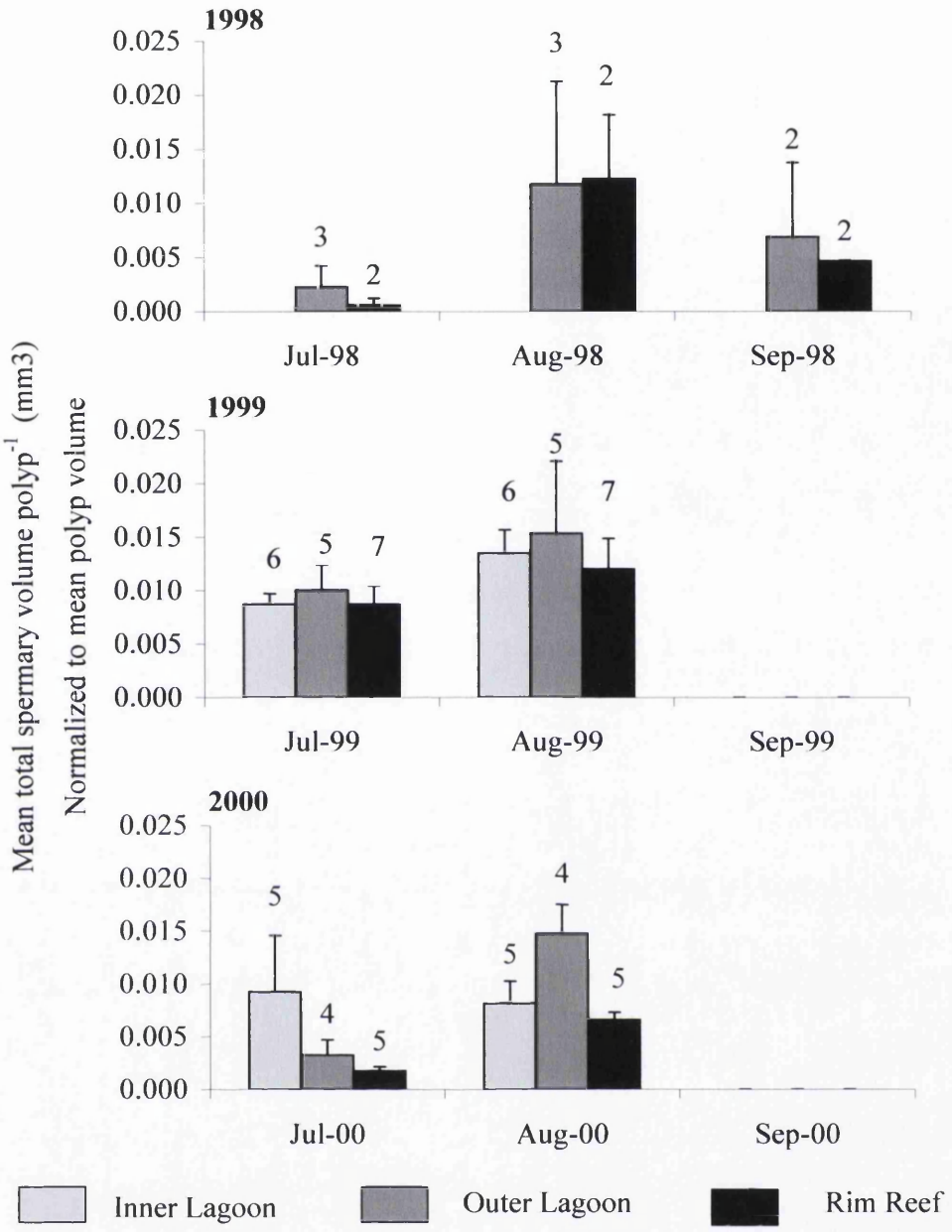


Figure 5.6: *Pseudoplexaura porosa* spermary reproductive effort combined from all colonies for each reef zone. Shown for the reproductive months of July-September 1998-2000. Spermaries were also present in October 1998 from the Rim Reef with a mean total spermary volume of 0.0003 mm^3 $polyp^{-1}$ (n=2). The number of colonies the mean for each reef zone is calculated from is shown above the bar. Error bars are +1 SE.

when there was a peak in spermary reproductive effort. Mature oocytes and spermaries persisted into September 1998 (Figures 5.5 and 5.6) and were also present in small numbers in October 1998 from two of the three female colonies and one of the two male colonies at the Rim Reef (not represented in Figures 5.5 and 5.6). Mean total oocyte volume (>500μm) in October 1998 was 0.004 mm^3 $polyp^{-1}$ and mean spermary volume was 0.0003 mm^3 $polyp^{-1}$. In contrast, there were no mature oocytes or spermaries found in September and October from any of the reef zones in 1999 and 2000, despite the increase in the number of colonies sampled per reef zone and the inclusion of the Inner Lagoon reef zone. Mature oocytes and spermaries were present earlier in the year, by July, and persisted through August each year (Figures 5.5 and 5.6).

Table 5.3: One-way ANOVA with repeated measures to examine for differences between *Pseudoplexaura porosa* reproductive effort of female (A) and male (B) colonies (arc sine transformed) across the three reef zones (Inner Lagoon, Outer Lagoon and Rim Reef) and the months of spawning (July and August) in 1999 and 2000. One level of nesting was used to examine for differences between the replicate reef sites within the zones (reefs within zone, and reefs within month; oocyte data run separately as it was necessary to omit July 1999 as only one site was monitored at the Outer Lagoon zone). See section 5.3.3 for details of the ANOVA design.

A: Female colonies

Oocyte: between subjects (zone)

Level	SS	df	Ms	Fs	P
Zone	1.871	2	0.936	1.313	0.292
Error	13.539	19	0.713		

Oocyte: within subjects (zone)

Level	SS	df	Ms	Fs	P
Month	3.900	2	1.950	11.727	0.000
Zone within month	0.352	4	0.088	0.529	0.715
Error	0.632	38	0.166		

Oocyte: intra-zone variation (reefs within zone)

Level	SS	df	Ms	Fs	P
Reefs within zone	1.486	3	0.495	0.695	0.566
Error	13.539	19	0.713		

Oocyte: intra-zone variation (reefs nested within zone and within months)

Level	SS	df	Ms	Fs	P
Reefs within month	0.579	6	0.097	0.581	0.743
Error	0.632	28	0.166		

B: Male colonies

Spermary: between subjects (zone)

Level	SS	df	Ms	Fs	P
Zone	0.221	2	0.110	1.956	0.184
Reefs within zone	0.358	3	0.119	2.115	0.152
Error	0.676	12	0.056		

Spermary: within subjects (zone)

Level	SS	df	Ms	Fs	P
Month	1.388	3	0.463	8.717	0.000
Zone within month	0.124	6	0.021	0.389	0.881
Reef within month	0.254	9	0.028	0.531	0.842
Error	1.911	36	0.053		

There was no significant difference in the oocyte or spermary reproductive effort from the *Ps. porosa* colonies between the replicate reefs within the zones (reefs within zones, P= 0.566 for oocytes; P= 0.152 for spermaries, Table 5.3) or in each spawning month and the reefs within the zones (reef nested within zone and month, P= 0.743 for oocytes; P= 0.842 for spermaries, Table 5.3). The mean oocyte and spermary volume from the replicate reef sites could therefore be combined to give the *Ps. porosa* reproductive effort per reef zone. The overall oocyte and spermary reproductive effort from the *Ps. porosa* colonies at all the reef zones combined for each spawning month was significantly different between the months (month, P= <0.001 for oocytes; P= <0.001

for spermaries, Table 5.3). The post-hoc test revealed that this was caused by the smaller overall reproductive effort of both female and male colonies in July 2000, the oocyte data being significantly different from August 1999, and the spermary data was significantly different from all other months (Appendix 5.8). There were no significant differences between the reproductive effort of the *Ps. porosa* colonies from each reef zone combined over the spawning months (zone, P= 0.292 for oocytes; P= 0.184 for spermaries, Table 5.3). However, the post-hoc test did show the mean oocyte volume from the Inner Lagoon to be significantly greater than that of the Outer Lagoon and Rim Reef colonies (Appendix 5.8). Combining the reproductive effort of the *Ps. porosa* colonies in each reef zone showed no significant difference between the spawning months (zone within month, P= 0.715 for oocytes; P= 0.881 for spermaries, Table 5.3).

The effect of temperature

Inter-annual variation in the seawater temperature profiles was examined as a possible factor affecting the differences in gamete maturation and spawning of *Pseudoplexaura porosa* colonies over the summer months of 1998-2000. The fluctuations in seawater temperature at the three zones of the Bermuda North Lagoon over the study years are discussed in detail in Chapter 2 and are shown in Figure 5.8. The results of the ANOVA (Table 5.3) showed no significant difference in *Ps. porosa* reproductive effort at the three reef zones, although reproductive effort varied significantly between the spawning months. The seawater temperature prior to spawning affects the timing of gamete maturation, the ultimate cue for gamete release being the lunar cycle (section 1.5, Chapter 1). The timing of each full moon differs from year to year, and so rather than the average temperature for each month, the average temperature for the 30 days prior to each sample date around the full moon was calculated for the temperature affecting the colonies prior to each spawning month. There is a positive relationship between colony reproductive effort and the average seawater temperature for the preceding month for both oocyte and spermary production (Figure 5.7). These relationships were significant at the 5% probability level (Product-moment correlation coefficient on arc sine transformed data; oocyte volume P= 0.021, r= 0.572, n= 16; spermary volume P= 0.001, r= 0.688, n= 19).

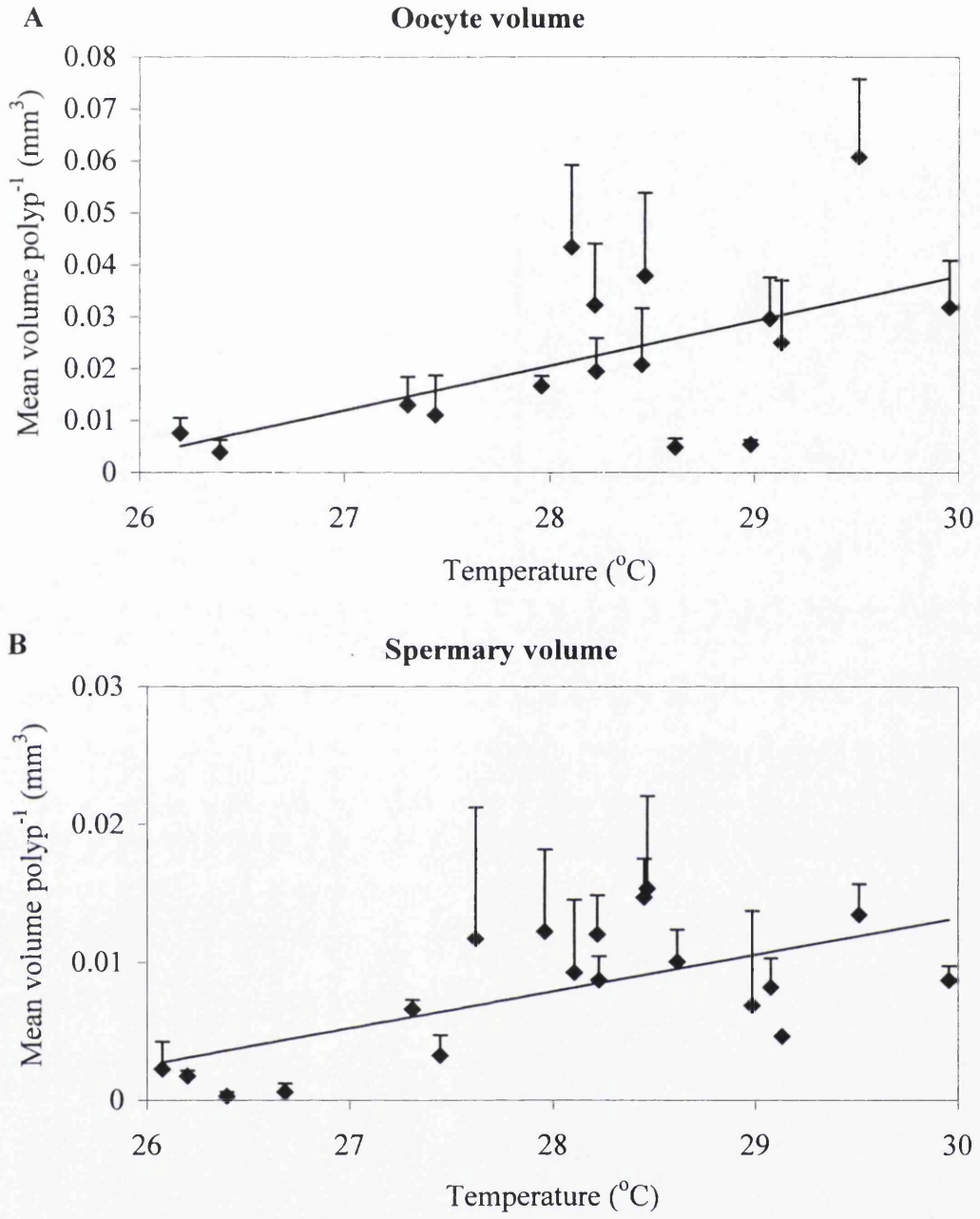


Figure 5.7: The relationship between seawater temperature and *Pseudoplexaura porosa* reproductive effort for female colonies (A) and male colonies (B). All reef zones are shown for July-September 1998-2000 and samples were collected just prior to spawning. October is included in 1998 only. Water temperature is the average for the lunar cycle (30 days) preceding the new moon for each month. Error bar is +1 standard error. Trendlines are linear regressions; see text for correlation analysis on transformed data.

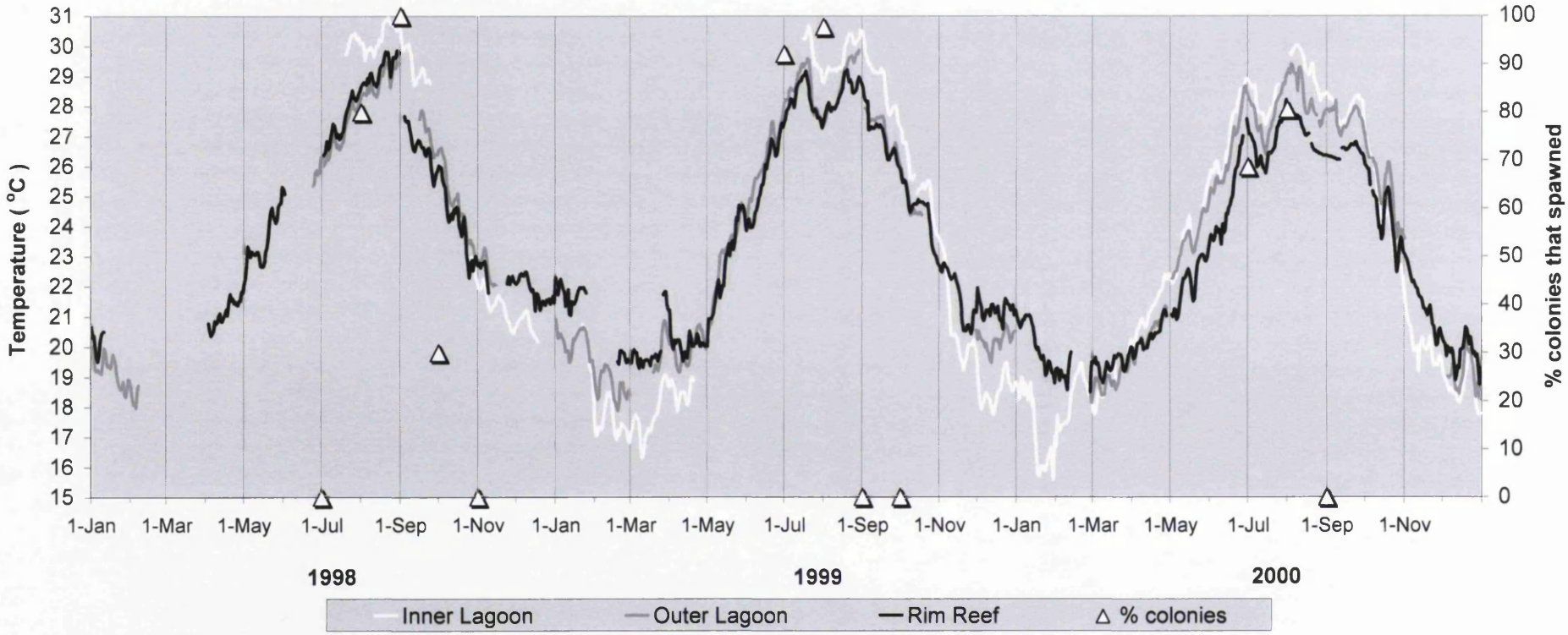


Figure 5.8: The percentage of *Pseudoplexaura porosa* colonies that spawned combined from the reef zones of the Bermuda North Lagoon plotted with the average daily seawater temperature for each reef zone over 1998-2000. Gaps in the temperature data are when data loggers were not deployed or malfunctioned. See Appendix 4.1 for the number of colonies sampled each month.

A further measure of reproductive effort is the percentage of *Ps. porosa* colonies that spawned each month, and this is related to the annual seawater temperature profiles in Figure 5.8. The summer of 1998 was relatively hot, whereas 1999 had moderate seawater temperatures and 2000 was comparatively cool (section 2.4, Chapter 2). The *Ps. porosa* reproductive season began later in 1998 as the seawater temperature in this year was slow to rise and the average temperature for the month prior to the July full moon reached between 26-26.7°C, dependent on the reef zone. Gamete maturation proceeded over July, and spawning occurred from 80% of the colonies from all the reef zones combined in August 1998 when average temperatures over the preceding month were >26.6°C (Figure 5.8). During the relatively hot summer of 1998, the temperature remained above 29°C for the majority of August. All colonies contained mature gametes for a successive spawning cycle in September 1998, with a small reproductive effort extending into October 1998.

In 1999, the seawater temperature rose earlier in the year, and average temperatures before the July 1999 full moon were already 28-30°C (dependent on the reef zone). Gamete maturation of the *Ps. porosa* colonies was advanced compared to 1998, beginning in July 1999 and leading to a spawning event that included 92% of the colonies after the July full moon (Figure 5.8). The 1999 reproductive season was shorter and only lasted through a second spawning event into August 1999. The average temperatures for the preceding month remained between 28 and 30°C and 98% of the colonies spawned. The temperatures in 1999 were moderate compared to 1998, exceeding 29°C only during short peaks in the summer months, and the maximum temperature from the Rim Reef was 0.7°C lower than in 1998. The average temperature over the month before the September full moon was approximately 1.5°C lower than that in September 1998 at the Outer Lagoon and Rim Reef zone and, in contrast to 1998, no spawning took place in September 1999.

In comparison, 2000 was a cool summer, although the timing of the temperature increase in the early summer was comparable to that of 1999. Gamete maturation therefore also occurred in July 2000, as the average temperatures for the month prior to the full moon were 27-28°C, and spawning occurred from 69% of the colonies (Figure 5.8). An exception is the Rim Reef in July 2000 when a small reproductive effort

occurred from the colonies even though the average temperature over the previous month was below 27°C. The cool summer temperatures did not exceed 29°C at the Rim Reef and only reached above 29°C in the Outer Lagoon and Inner Lagoon for short periods. Spawning occurred over a second month in August, although reproductive effort was lower than in August 1999 and only 81% of the colonies contained mature gametes. Spawning did not take place from the monitored colonies in September 2000.

5.5 Discussion

The coral populations of Bermuda are subjected to sub-optimal temperature conditions for several months of the year as the reef zones experience temperatures of 29-31°C in summer but only 15.5-18°C in winter. The breeding seasons for the brooding scleractinian *Porites astreoides* and the broadcasting gorgonian *Pseudoplexaura porosa* in Bermuda occur over a brief period of the year. The planula release of *Po. astreoides* and the gamete release of *Ps. porosa* (with the exception of 1998; see below) are both maximal in July and August. The reproductive season of these two species is restricted in Bermuda compared to conspecifics in the Caribbean and this can be explained in terms of the timing of the optimal temperature range for reproduction at the different latitudes. In the Florida Keys, planula release from *Po. astreoides* colonies occurred in April and May with planulation continuing through the summer until September from a small percentage of colonies (McGuire, 1998). A further decrease in latitude in the Caribbean and the reproductive season of *Po. astreoides* is extended, being from January until September in Puerto Rico (Szmant, 1986) and year round in Panama and Jamaica (Chornesky and Peters, 1987; Soong, 1991). McGuire (1998) related the shortening of the reproductive season of *Po. astreoides* in Florida relative to the wider Caribbean to differences in the annual temperature range, which is 6.5 °C in Florida (McGuire, 1998), compared to ~4 °C in Puerto Rico and Jamaica (CARICOMP, 1997). In Bermuda, the annual temperature range is greater still, being 11-15°C. A narrow temperature range at lower latitudes will encourage reproduction to occur year round, whereas a wide annual temperature fluctuation will cause seasonality in the

reproductive cycle as defined by Orton's (1920) rule stating that marine animals reproduce at a defined temperature, or change in temperature, that is species-specific. Increasing seasonality to the breeding patterns of several Pacific brooding species also occurs with increasing latitude (Harriott, 1983; Stoddart, 1985; Kojis, 1986b; Yamazato *et al.*, 1991; Hayashibara *et al.*, 1993; Tanner, 1996).

McGuire (1998) confirmed the controlling effect of seawater temperature on the timing of the reproductive season of *Porites astreoides* in Florida by documenting inter-annual variation in the maximum numbers of colonies releasing planulae in accordance with the different yearly seawater temperature profiles. The main reproductive effort of colonies in Florida occurred each year when the seawater temperature from the preceding lunar cycle was between 24.5 and 27.5°C. Planula release was greatly reduced once the seawater temperature reached maximal values of 29-30 °C. At the lower latitude of Jamaica, where *Po. astreoides* planulation occurs year round, the maximum number of planulae were found in the colonies in April when seawater temperatures were still increasing (Chornesky and Peters, 1987). The annual temperature range in Jamaica is smaller than in Florida, and the average seawater temperature is therefore above 28°C by April, and the maximum temperature around 30°C reached five months later (CARICOMP, 1997). Maximum reproductive effort of *Po. astreoides* in Jamaica therefore also occurs before the seawater temperature maximum, but planulation is extended as temperatures remain favourable. In Bermuda, there is also a relationship between seawater temperature and *Po. astreoides* reproductive effort. Planula release primarily occurred when water temperatures for the preceding lunar cycle were between 26.5 and 28.5°C. This is slightly higher that the optimal temperatures in Florida, but still below the summer maximum. The lower winter temperatures in Bermuda lead to a more rapid temperature increase over the spring and early summer than in Florida. Thus, the average temperature prior to the maximum is slightly higher in Bermuda and favourable monthly temperatures for reproduction occur just one month before the maximum temperature.

The spawning of *Pseudoplexaura porosa* in Panama extended over 3-4 months, from June until August with a small reproductive effort in September (Kapela and Lasker, 1999). The reproductive season of *Ps. porosa* is somewhat restricted at the higher

latitude of Bermuda, occurring over 2 months (with the exception of 3 months for a low percentage of colonies in 1998). The contrast in the duration of the reproductive season between Bermuda and the central Caribbean is not as great for the broadcasting gorgonian *Ps. porosa* as it is in the brooding scleractinian *Po. astreoides*. Scleractinian broadcasting species also show less latitudinal variation in reproduction compared to scleractinian brooding species (Chapter 7). The spawning of *Pseudoplexaura porosa* in Bermuda (this study) and in Panama (Lasker *et.al.*, 1996; Kapela and Lasker, 1999) occurring just prior to and during the time of maximum seawater temperature, which is in accordance with data from other gorgonian species studied (Grigg, 1979; Goldberg and Hamilton, 1974; Brazeau and Lasker, 1989; Coma *et.al.*, 1995a).

In contrast to the reproductive season of *Po. astreoides*, there was no discernible minimum or maximum temperature that could be associated with the continuation or cessation of gamete maturation for *Ps. porosa*. However, temporal separation in the month of spawning of *Ps. porosa* occurred over the study years in association with the different annual seawater temperature patterns. The gametes of *Ps. porosa* take four to six weeks to develop to maturity (Chapter 4), and this primarily occurred when the average seawater temperature over the maturation period was greater than 27°C. The rise in seawater temperature was delayed in the early summer of 1998 compared to the following two years of study. Consequently gamete maturation was not initiated until a month later and spawning delayed until August 1998, compared to the earlier spawning beginning in July of 1999 and 2000. The dependence of gorgonian gamete maturation on seawater temperature is supported by a study on the gorgonians *Muricea californica* and *M. fruticosa* off the West Coast of the Americas. Deeper populations spawned one month later than conspecifics living inshore in accordance with the delay in the timing of maximum seawater temperature (Grigg, 1979). A similar delay in spawning to coincide with maximum seawater temperature has been documented to occur for some scleractinian broadcasting species. The offshore reefs of the central Great Barrier Reef are cooler and reach maximum seawater temperatures after the inshore reefs of a similar latitude, and this causes a delay of one month in the timing of the same scleractinian coral species (Harrison *et al.*, 1984; Willis *et al.*, 1985). The mass spawning at the high latitude Solitary Islands in Eastern Australia occurs 2-5 months after the spawning of the Great Barrier Reef, coinciding with the lag in the maximum seawater temperature

(Wilson and Harrison, 1997; see review in Chapter 1). In 1999 and 2000 the tagged *Ps. porosa* colonies contained mature gametes over just one or two months. The extension of spawning into a third month did occur with a weak reproductive effort from a small number of the Rim Reef colonies in October 1998. This may have been an effect of the anomalous hot temperatures at that reef zone over the summer of 1998 allowing the extension of the period of gamete maturation.

Seasonal changes in daylength (photoperiod) are also extreme at high latitude reefs and for some species it is not clear whether either gametogenesis and spawning or planula release are initiated by increasing daylength, or rising seawater temperature over the summer period (Kruger and Schleyer, 1998; Chapter 1). It is likely that the two abiotic factors have a synergistic control of the breeding season on the high latitude reefs of Bermuda. Other factors may also exert a stronger proximate or selective force on the timing of reproduction and thereby over-ride the control of temperature, such as favourable conditions for the survival of the larvae or the influence of temporally predictable weather or oceanographic patterns (Chapter 1). The effect of the environmental cues on the timing of the reproductive season, however, is species-specific. For example, colonies of *Favia fragum* in Bermuda release planulae over an extended period of the year, and planulate as early as February when seawater temperature and photoperiod are at their minimum (Kuffner and Smith, unpub. data; see Chapter 7).

In addition to the controlling effect of the annual temperature profile on the reproductive season of *Porites astreoides* and *Pseudoplexaura porosa*, there is also a relationship between reproductive effort and average seawater temperature, which differs between the species. The cooler summer temperatures in 2000 were more favourable for *Po. astreoides* reproduction and a greater number of planulae were released compared to 1999. There is a significant negative relationship between reproductive effort and temperature for this species. In contrast, the cooler temperatures in 2000 corresponded with a lower reproductive effort in terms of gamete production of *Ps. porosa* colonies, and there is a significant positive relationship between reproductive effort and increasing temperature. In addition, five tagged *Ps. porosa* colonies did not produce any mature gametes over the hot summer of 2000, whereas all colonies were reproductively active in 1998 and 1000 (section 4.5.4, Chapter 4). The contrasting

relationship between reproductive effort of *Po. astreoides* and *Ps. porosa* and seawater temperature is, in part, a reflection of the different optimum temperature for reproduction, which occurs below the seawater temperature maximum for *Po. astreoides* and at the maximum temperature for *Ps. porosa*. However, the controlling effect of temperature on the reproductive effort of *Po. astreoides* was also shown within the reef zones of the Bermuda platform, especially over the month of August. The 1-3°C difference in summer seawater temperature between the August new moon of 1999 and 2000 is significantly correlated to an increased reproductive effort August at each reef zone. The potential for small fluctuations in seawater temperature to affect reproductive effort is supported by the work of Jokiel and Guinther (1978), who reported that a change of just 1°C from optimum significantly diminished the reproductive effort of *Pocillopora damicornis* colonies. *Po. astreoides* reproductive effort is therefore correlated to temperature variation occurring on both a spatial scale, across the Bermuda North Lagoon within a month, and also a temporal scale, within and between the years of study. There was no significant difference in reproductive effort of *Ps. porosa* colonies among the different reef zones and *Ps. porosa* reproductive effort was only correlated to temperature variation occurring on a temporal scale.

As in most animals, energy budgets in corals are limited and after the allocation of energy to essential metabolism, there is a trade-off between the use of energy for growth and calcification versus reproduction. Colonies of *Pocillopora damicornis* which produced brooded planulae had a slower growth rate than those colonies in the same population which only produced sperm, indicating brooding to be the more 'costly' process (Ward, 1995). Developmental times for reproduction are also greater in species with a brooding mode of reproduction, because there is a further 2-3 week period for planula development in addition to the period of after gamete maturation over one to two months (Szmant-Froelich *et.al.*, 1985; Chapter 3 for *Po. astreoides*). The high metabolic cost of a brooding reproductive cycle compared to a broadcasting reproductive mode will therefore make the former more susceptible to temperature changes outside the species defined optimum. Differences in reproductive mode have also been attributed to variations in the response of coral species to nutrient enrichment. For example, reproduction of the broadcasting *Montipora capitata* in Hawaii showed

few significant changes after ammonium enrichment, whereas planulation of the brooding species *Pocillopora damicornis* ceased with the same level of exposure (Cox and Ward, 2002).

In the present study, the species differ in their taxonomic sub-class in addition to their reproductive mode. An interesting study would be to examine the effect of temperature on a broadcasting scleractinian species in Bermuda, in contrast to the brooding reproductive mode of *Po. astreoides*. This would address the question of whether contrasting reproductive modes or the different energy budgets of scleractinian versus gorgonian species is driving the observed differences in the relationship between temperature and reproductive effort in *Po. astreoides* and *Ps. porosa*. The broadcasters *Diploria strigosa*, *D. labyrinthiformis*, *Montastrea cavernosa* and *M. franksi* are known to synchronously spawn gametes on the third quarter moon phase of one or two months between July until September (Wyers *et al.*, 1991, *M. franksi* as *M. annularis*). Broadcast spawning therefore occurs at the time of maximum seawater temperature in Bermuda, varying between years, depending on the timing of the full moon within the month and the yearly temperature profile. However, there is no information on the effect of temperature on reproductive effort for these species.

There was no inter-zone variation in overall *Ps. porosa* reproductive effort among the reef zones. In contrast, the *Po. astreoides* colonies released a lower overall number of planulae at the Inner Lagoon reef zone. The temperature at the Inner Lagoon increased over 28°C in July and August 2000, reaching a greater summer maximum than the other reef zones, thereby suggesting temperature inhibition of reproductive effort of the *Po. astreoides* colonies. However, other factors such as the increased sedimentation rates may act to reduce planula production at the Inner Lagoon (Chapter 3). Kojis and Quinn (1984) showed that sedimentation and turbidity synergistically decreased the fecundity of *Acropora palifera* as a result of reduced light levels and an increase in energy consumption related to sediment removal. Reduced light levels through pollution also caused depressed planulae development and maturation in *Porites porites* (Tomascik and Sander, 1987). There is also the possibility that the reproductive effort from the Inner Lagoon colonies is an underestimate, and that planula release occurred outside the monitored period due to a reduction there in the degree of lunar synchrony to

planulation (Chapter 3). Removal of the Inner Lagoon data when examining the relationship between reproductive effort and the average temperature for the preceding lunar cycle still shows a negative trend, although the correlation is not significant. Wave energy is another environmental factor that changes from inshore to offshore of the Bermuda platform (Chapter 2). The lower wave energy inshore at the Inner Lagoon and enclosed basins is more favourable for the growth of coral species (Logan and Tomascik, 1991) and also modifies the growth form of *Po. astreoides* and *Ps. porosa* compared to conspecifics offshore (Chapter 3 for *Po. astreoides* and Chapter 4 for *Ps. porosa*). Wave energy also varies inter-annually with the frequency of winter storms. However, the gradient of decreasing wave energy towards the Inner Lagoon is not correlated to the observed relationship of decreased reproductive effort of *Po. astreoides* colonies within the Bermuda platform or between the years. Food levels also differ across the Bermuda platform, but are higher inshore compared to the offshore reefs (Beers and Herman, 1969; Morris *et al.*, 1977). The availability of foods is therefore not thought to be a limiting factor on coral reproduction in Bermuda as increased food levels occur inshore where there is a decreased reproductive effort.

5.6 Summary

Release of *Porites astreoides* planulae and spawning of *Pseudoplexaura porosa* in Bermuda occurred over a narrow period in the summer months of July and August 1999 and 2000, and a small number of planulae were released from *Po. astreoides* colonies from offshore reef zones in September each year. *Ps. porosa* spawning was delayed and extended over the hot summer of 1998 (*Po. astreoides* not studied) and occurred from August until October. The duration of the breeding season of these species increases with a decline in latitude through Florida to the central Caribbean, in association with a decrease in annual temperature range towards the equator. Planula release in *Po. astreoides* occurs just before the seawater temperature maximum in the summer, whereas *Ps. porosa* spawning occurs at the peak temperatures. The association of the reproductive season with a preferred temperature range caused a variation in the timing and magnitude of reproductive effort in accordance with the different annual

temperature profiles over the study period. In addition, seawater temperature had a contrasting effect on the reproductive effort of the brooding scleractinian *Po. astreoides* and the broadcasting gorgonian *Ps. porosa*. Over the relatively cool summer of 2000, *Po. astreoides* reproductive effort was greater than that of the colonies monitored during the moderate temperatures of 1999. In contrast, *Ps. porosa* reproductive effort decreased in the cool summer of 2000 when compared to 1999, and reproductive effort was greatest over the relatively hot summer of 1998. The different allocation of energy to reproduction by a brooding versus broadcasting species, or between a scleractinian versus gorgonian species, may be responsible for to the observed contrasting effect of temperature on colony reproductive effort between the study species. More research on the environmental effects influencing the reproductive cycles of coral species with opposing reproductive modes and from disparate orders is needed to understand further the controlling role of temperature on anthozoan reproductive cycles.

Chapter 6: Sexual reproduction of *Madracis mirabilis* (Scleractinia: Pocilloporidae) in Bermuda

6.1 Introduction

The reproductive mode of scleractinian corals is classically described as either the broadcast spawning of gametes for external fertilisation or the internal fertilisation and brooding of planulae (for reviews see Fadlallah, 1983a; Szmant, 1986; Harrison and Wallace, 1990; Chapter 1). Combining these reproductive modes with sexuality led to the definition of four types of sexual pattern in scleractinian corals: hermaphroditism combined with either broadcasting or brooding; and gonochorism combined with either broadcasting or brooding (Szmant, 1986). Subsequently, it has been discovered that these classical modes show variation among some species, specifically in the length of the brooding period.

Montastrea cavernosa and *Stephanocoenia intersepta* were originally described as broadcast spawners (Szmant, 1991; Hagman *et al.*, 1998a). However, recent observation on the spawning of these massive scleractinian species suggests that internal fertilisation actually takes place. The fertilised eggs are not brooded internally but instead are released at the zygote or embryo stage (Hagman *et al.*, 1998a; Hagman *et al.*, 1998b). Internal fertilisation followed by the release of zygotes and external development of planulae is also described for the scleractinian *Eusmilia fastigiata* (de Graaf *et al.*, 1999). A recent study on the *Madracis* species complex in Curaçao in the Caribbean presented further evidence of this proposed new strategy of internal fertilisation followed by zygote or embryo release in scleractinians (Vermeij *et al.*, in review; Vermeij *et al.*, 2003). Vermeij *et al.* (in review) presented the term "quick-releasing" to describe this reproductive mode that is neither brooding nor broadcasting. Despite this recent work on the reproductive cycle of corals, the mode of reproduction is currently unknown for over 1100 scleractinian species (P. Harrison, pers. comm.). This lack of knowledge, in addition to the observation that two documented broadcast spawning species may actually undergo internal fertilisation and the "quick-releasing" of zygotes, implies that this reproductive mode may be more widespread.

The growth in research into coral reproduction has not only discovered variations in previously defined reproductive modes, but has shown that scleractinian reproductive patterns are not stable across geographic locations (for review see Chapter 1). For example, populations of *Pocillopora damicornis* brood planulae in the Central Pacific (Richmond and Jokiel, 1984; Richmond, 1985; Richmond, 1987) and the Great Barrier Reef (Harriott, 1983), whilst conspecifics broadcast gametes in the Eastern Pacific (Glynn *et al.*, 1991). Similarly, populations of *Pocillopora verrucosa* are brooders at Enewetak Atoll (Stimson, 1978) but are broadcast spawners in the Red Sea (Shlesinger and Loya, 1985) and off South Africa (Kruger and Schleyer, 1998). A further example is *Acropora humilis*, which broods planulae in the Central Pacific and spawns gametes in the Red Sea and the Great Barrier Reef (Richmond and Hunter, 1990). In addition, colonies of at least one species, *Goniastrea aspera*, are known to individually undergo both broadcast spawning of gametes and sexual production of planulae (Sakai, 1997, in Okinawa, Japan). Populations of *G. aspera* in the Palau Islands release planulae (Harrison *et al.*, 1984) and on the Great Barrier Reef this species is a spawner (Babcock, 1984). Therefore, it is necessary to study the same species across geographic locations before broad generalisations as to reproductive strategies can be made.

This study determined the reproductive cycle of *Madracis mirabilis* in Bermuda, particularly the reproductive mode. *M. mirabilis*, commonly known as the yellow pencil coral, is widely distributed throughout the Caribbean, Gulf of Mexico and Bermuda (Veron, 2000). The genus *Madracis* (Milne-Edwards and Haime, 1849) was originally classified into the scleractinian family Pocilloporidae. Veron (2000) replaced the genus to the family Astrocoeniidae on the basis of columella characteristics. This taxonomic change has not been supported by the evolutionary, ecological and reproductive studies of the genus by Vermeij (2002), who re-assigned the genus back to the Pocilloporidae. The five common reef-building species in the Caribbean are *M. mirabilis* (Lyman, 1859), *M. decactis* (Lyman, 1859), *M. formosa* (Wells, 1973), *M. pharensis* (Heller, 1868), and *M. senaria* (Wells, 1973). The species *M. mirabilis* and *M. decactis* were originally described from Bermuda (see Sterrer, 1986). However, the species status of *M. decactis* is doubtful as the phylogenetic study on the genus by Diekmann *et. al.* (2001) concluded that only *M. mirabilis* and *M. senaria* are genetically separate species, while the closely related *M. decactis, M. formosa* and *M. pharensis*

comprise a paraphyletic complex. This division of the genus to two distinct species and a set of morphospecies is supported by the studies of Vermeij (2002). The characteristics organising the *M. decactis-M. formosa-M. pharensis* species complex separated a new species type, *M. carmabi* (Vermeij, in review).

Colonies of *M. mirabilis* in Bermuda are differentiated from *M. decactis-M. formosa* in that they form flat or hemispherical aggregations of branches, or can form continuous beds of small tan to yellowish branches. A common skeleton usually connects the branches of a colony but the tissue at the bases of the branches recedes and often becomes colonised by algae and sponges. Colonies show a high degree of phenotypic plasticity in corallite characteristics, branch diameter, branch length, and spacing of the branches dependent on environmental variables (Bruno and Edmunds, 1997; Sebens *et al.*, 1997; Bruno and Edmunds, 1998). The corallites are ~1.5 mm in diameter (Veron, 2000) and the polyps are generally extended during both day and night. Gametogenic processes of *M. mirabilis* were originally documented by Delvoye (1988). A more recent study of the reproductive cycle of the six sympatric species of *Madracis* has been presented by Vermeiji *et al.* (in review; 2003). All of these studies were located in Curaçao in the Southern Caribbean. The colonies of *M. mirabilis* here were gravid from June until November, the development of gametes being correlated with increasing and maximum seawater temperature. The colonies are hermaphroditic at both the colony and polyp level, with Delvoye (1988) describing gonochoric mesenteries, whereas the more extensive study of Vermeij *et al.* (in review) found both gonochoric and hermaphroditic mesenteries. Neither embryos nor planulae were found in the histological sections (Delvoye, 1988; Vermeij *et al.*, 2003), although early-stage planulae were collected *in situ* from the colonies of all of the *Madracis* species, with abundance increasing over September to November (Vermeij and Bak, 2002; Vermeij *et al.*, 2003). The abundance of planulae collected from *M. senaria* colonies was greatest over the third quarter moon phase, although there was no lunar periodicity to planula abundance of the other *Madracis* species.

The reproductive cycle and mode of *M. mirabilis* is similarly investigated in the present study to examine sexuality, fecundity, gametogenesis, lunar periodicity and the occurrence of the proposed "quick-releasing" strategy, and also to document the timing

of the reproductive season of this species in the sub-tropical reef environment of Bermuda.

6.2 Objectives

This study addresses the following questions on the reproduction and abundance of *Madracis mirabilis* in Bermuda:

1. What is the timing and duration of gametogenesis of *M. mirabilis* in Bermuda?
2. What is the sexuality and fecundity of *M. mirabilis* colonies?
3. What is the reproductive mode of *M. mirabilis* in Bermuda?
4. Is there lunar periodicity to gamete and/or planulae release?

6.3 Methods

6.3.1 Distribution and abundance of *Madracis mirabilis* across the Bermuda platform

A preliminary observational survey was carried out to determine the abundance of *Madracis mirabilis* at the reef zones of the Bermuda platform (see Chapter 2 for a description of the Bermuda reef zones). This species flourishes inshore on the patch reefs of the Inner Lagoon. Distribution is patchier at the Outer Lagoon but extensive colonies were found at several reef sites. Occurrences were rare at the outer Rim Reef zone, which encircles the Bermuda platform, and only very small colonies were sparsely found along the North and South shores. Sporadic occurrence of *M. mirabilis* also occurs at the deeper terrace reef of the North and South shores and these populations can be locally abundant. Replicate study sites were chosen to represent both the Inner Lagoon and the Outer Lagoon (Figure 2.1, Chapter 2), where *M. mirabilis* is common,

to examine whether the variable environmental conditions at the two reef zones affect the timing or duration of the reproductive cycle. The rare occurrence of *M. mirabilis* offshore at the Rim Reef zone was investigated by determining the survival rate of transplanted branches in an exploratory experiment, the results of which are presented in Appendix 6.1 and discussed in relation to the local environmental factors. The main Terrace was not included as these deeper reefs are frequently inaccessible from rough seas and time constraints.

6.3.2 Histological Examination

Branches of *Madracis mirabilis* were collected for histological examination of reproductive structures from replicate sites at the Inner Lagoon and Outer Lagoon over the summer months of 1998 and 1999 (Table 6.1). In 1998, samples were collected at six intervals between July to September. The sampling dates were more extensive in 1999, with samples collected before the full moons of May and June and then collected over eight intervals between July and September, and a final sample was collected before the full moon of October 1999. A sample in 1998 consisted of one branch from each of two separate colonies. In 1999, this was increased to one branch from each of three separate colonies from July to September, with one branch from two colonies sampled in June and October and one branch from only one colony sampled from each site in May. Additional branch samples were collected from a total of 12 colonies during August 1998 and 12 colonies over August 1999 (Table 6.1) and histological slides were made to determine sexuality and to look for the presence of embryos or larvae. Measurements of fecundity and gamete size were not made from these additional samples.

Collection was made by SCUBA and branches were dislodged at the colony base with pliers. All samples were immediately fixed in 9% seawater formalin for 36 hours before being transferred to 10% formic acid for decalcification. The formic acid was changed daily and the process was complete between 5-7 days. All tissue samples were then washed and preserved in 70% EtOH. A razor blade was used to cut a rectangle of

Table 6.1: *Madracis mirabilis* sampling schedule for histological examination of reproductive structures over 1998 and 1999. Replicate study sites were sampled from the Outer Lagoon: Crescent A and Crescent B, and the Inner Lagoon: Tynes West and Tynes East (Figure 2.1, Chapter 2). One branch was sampled per colony. Additional samples were collected from each reef zone over August each year for sex determination (gamete counts were not made).
New moon= lunar day 0, full moon= lunar day 15; and 3/4 moon= lunar day 22

		Number of colonies sampled					
		Outer Lagoon			Inner Lagoon		
1998	Lunar day	Crescent A	Crescent B	Additional	Tynes West	Tynes East	Additional
6-Jul-98	12	2	2		2	2	
20-Jul-98	26				2	2	
21-Jul-98	27	2	2				
3-Aug-98	11				2	2	
5-Aug-98	13	2	2				
13-Aug-98	21	2	2	6	2	2	6
21-Aug-98	0	2	2				
24-Aug-98	3				2	2	
13-Sep-98	23	2			2	2	
1999	Lunar day	Crescent A	Crescent B	Additional	Tynes West	Tynes East	Additional
25-May-99	9	1	1				
27-May-99	11				1	1	
22-Jun-99	8	2	2				
25-Jun-99	11				2	2	
12-Jul-99	28				3	3	
13-Jul-99	0	3	3				
1-Aug-99	19				3	3	2
2-Aug-99	20	3	3	2			
12-Aug-99	1				3	3	2
13-Aug-99	2	3	3	2			
26-Aug-99	15	3	3	2			
27-Aug-99	16				3	3	2
30-Aug-99	19	3	3				
31-Aug-99	20				3	3	
3-Sep-99	23				3	3	
5-Sep-99	25	3	3				
9-Sep-99	0	3					
10-Sep-99	1				3	3	
26-Sep-99	17	3	3				
27-Sep-99	18				3	3	
12-Oct-99	4		2				
14-Oct-99	6				2	2	

15 polyps from the tissue and then this was dehydrated, cleared and embedded in paraplast. Serial cross sections were made at 8μm through the whole tissue from tentacles to base and sections were mounted on slides and stained with Mallory's Triple Stain (Humason, 1962; Grimstone and Skaer, 1972). The slides were examined using a compound microscope under the x10 and x20 lenses and measurements were performed using the ImagePro (Version4) Image Analysis package. Five polyps were randomly chosen from the slides of each branch sample and the number and size of all oocytes within those polyps measured at their greatest diameter across the nucleus. The same five polyps were also examined for spermary development. Spermaries lack a characteristic feature, such as the nucleolus of oocytes, which ensures measurement at the greatest diameter and avoids repeated counts. Thus, only the middle section of each slide (equating to intervals of 56μm) was observed to monitor spermary development and the number, size and developmental stage of spermaries recorded (as defined in Table 6.2).

Table 6.2: Classification of spermary stages.

Stage	Description
1	Small clusters of spermatogonia (not observed in the sections).
2	Spermaries of a variable size (35-45μm diameter) and surrounded by a thick mesoglea. Spermatocytes evenly distributed throughout spermary.
3	Large spermaries of variable diameter with central lumen. Prominent primary spermatocytes, darkly stained with large nuclei (3-4μm).
4	Spermaries same size as stage 3 and full of smaller spermatids (<2μm) and mature spermatozoa with tails.

6.3.3 Laboratory monitoring of spawning

The histological sections of *Madracis mirabilis* showed gamete size to be at a maximum over the summer months of August and September. *M. mirabilis* gravid branches were collected over these months in 1998 and then again in 2001 and monitored in aquaria

for gamete or planulae release. Samples were collected every 5-7 days over August and September of each year. The coral branches were replaced onto the reef and new samples collected on each sample date to avoid stress to the colonies from being held in aquaria and possibly compromising reproductive effort. The sample size on each date in 1998 was five branches from each of five random colonies from the Inner Lagoon sites, and in 2001 this was increased to ten branches from ten random colonies, five colonies from the Inner Lagoon and five colonies from the Outer Lagoon sites (Figure 2.1, Chapter 2). All branch samples were collected by SCUBA and then transported in coolers of seawater back to the outdoor wet bench facilities at BBSR.

Two methods were used to collect the gametes or planulae. In 1998, the branches from each colony were placed inside a clear Tupperware container (2.5litre; 18 cm x 12 cm deep) with a water flow inlet to the base. To maintain a constant seawater temperature, Velcro strips attached the pots to the base of a larger aquarium of running seawater. The container lids had a large window cut out which was covered in 50μm nitex screen to allow water flow whilst retaining any eggs or planulae. The planulae of *Madracis* colonies in Curaçao were primarily released at night (Vermeij *et.al.*, 2003). The lids were therefore removed during the daytime to promote water circulation to the colonies and reduce shading. Every few days the lids were replaced in the evening and the pots were removed the following morning and examined under a dissecting microscope. The colour and movement of any coral propagules were recorded.

The method used over the summer of 2001 was to keep the coral branches in individual clear plastic aquaria, which were all placed inside a larger aquarium of running seawater to maintain a constant temperature. Every few days the water in this larger aquarium was lowered in the evening to below the top of the individual aquaria holding the coral branches, so that any released propagules that night remained in the contained water of the smaller aquaria. Aerators maintained oxygen supply and water movement to the branches. The water was exchanged inside each aquarium on the evening prior to examination for coral propagules, so that only gametes or planulae released that night would be counted the following day. The water was siphoned out through a 50μm mesh in the morning, approximately 13-16hr later. The contents of the mesh were re-suspended in seawater and examined under a dissecting microscope. The colour and

movement of any coral propagules were recorded. The collections of branches in September 2001 were held in an aquaria of filtered seawater to investigate the possibility of contamination of planktonic eggs and larvae from the aquarium water supply. Suspected coral propagules from the collections in 2001 were examined as slide squash preparations under the compound microscope. Fluorescent microscopy was used to detect the presence of zooxanthellae in the propagules.

6.3.4 *In situ* monitoring of spawning

Madracis mirabilis colonies were monitored *in situ* for spawning by the use of larval traps in the summer of 2001. The trap is modified from a design by Vermeiji *et. al.* (2003) and consists of a 20 cm length of tights (pantyhose leg) that was stretched to attach to a 15 cm diameter PVC ring at the base and a 0.5 litre inverted funnel at the top. The receptacle for the coral propagules was a 50 ml centrifuge tube. A hole was drilled through the cap that allowed it to fit over the neck of the funnel and it was secured in place. The tube was then inverted and screwed in place underwater leaving a few centimetres of air at the top for buoyancy. The PVC ring making the base of the trap was placed over a coral colony and held down by fishing weights. Nine traps were deployed at each of the Inner Lagoon sites (Tynes West and Tynes East; Figure 2.1, Chapter 2) on August 9th 2001. The traps were monitored every other day for two weeks and the centrifuge tubes changed *in situ*. The traps were moved to new colonies half way through the deployment period (one week) in case of stress effects to the colonies from reduced water flow and shading. The collected tube contents were examined under the dissecting scope and the colour and movement of any coral gametes or planulae were recorded and compared to the laboratory collections.

6.4 Results

6.4.1 Sexuality and fecundity

Madracis mirabilis has a short annual reproductive cycle in Bermuda with oocytes visible from June until September. Spermaries develop later than oocytes and were not recorded until August (Figure 6.1). Neither planulae nor developing embryos were found in any sections. A total of 159 branches were sampled for sex determination over the reproductive months of June to September in 1998 and 1999 (not including the last sample date in September 1999 when no gametes were found). Of these sampled branches, 45% contained no gametes in the sections examined. The maximum number of reproductive branches occurred in August (67%, n=96 combined from 1998 and 1999) when the majority of branches were hermaphroditic with only 6% of branches remaining female and 5% containing only spermaries (Figure 6.1). By September in each year, all reproductive branches were either hermaphroditic or male.

The sex of individual polyps varied among hermaphroditic branches. Of the colonies reproductive in August and September 1998 and 1999 from which gamete counts were made, 70% of individual polyps were hermaphroditic, 16% were male, 6% were female and 8% had no gametes (n=50 branches, 250 polyps). In hermaphroditic polyps, egg and sperm clusters were often intermingled in the same mesentery (see Figure 6.7C). The majority of individual polyps had fewer than 10 oocytes polyp^{-1} but the range was variable and the maximum was 186 oocytes polyp^{-1} and mean was 22.7 oocytes polyp^{-1} (Figure 6.2). The number of spermaries per polyp was undetermined as not all spermaries were measured.

6.4.2 Oogenesis

The smallest oocytes detected were in the mesoglea and had a diameter of 15-35μm (Figure 6.3A). The germinal vesicles were relatively large (~10μm) with a prominent nucleolus (G and N in Figure 6.3A). The vitellogenic cytoplasm of medium sized oocytes of 40-60μm diameter was more darkly stained, and contrasted the pale and

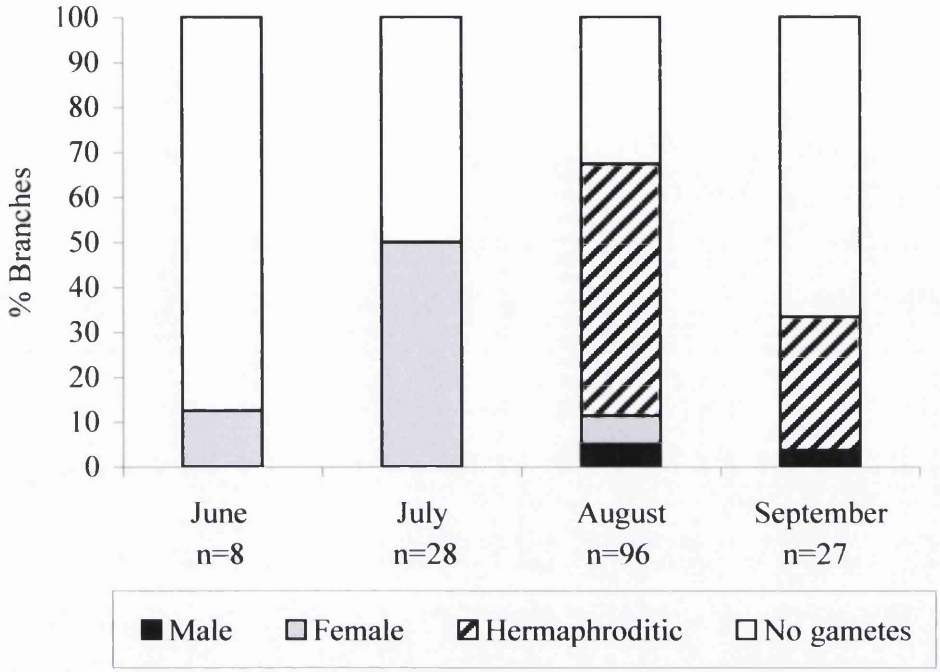


Figure 6.1: The monthly variation in the percentage of *Madracis mirabilis* branches that were male, female or hermaphroditic. Data from 1998 and 1999 have been combined and shown as a percentage of the total number of branches sampled in that month (shown as n on the x axis). Five polyps were examined from each branch. The last sample date in September 1999 was not included as all of the branches were non-reproductive.

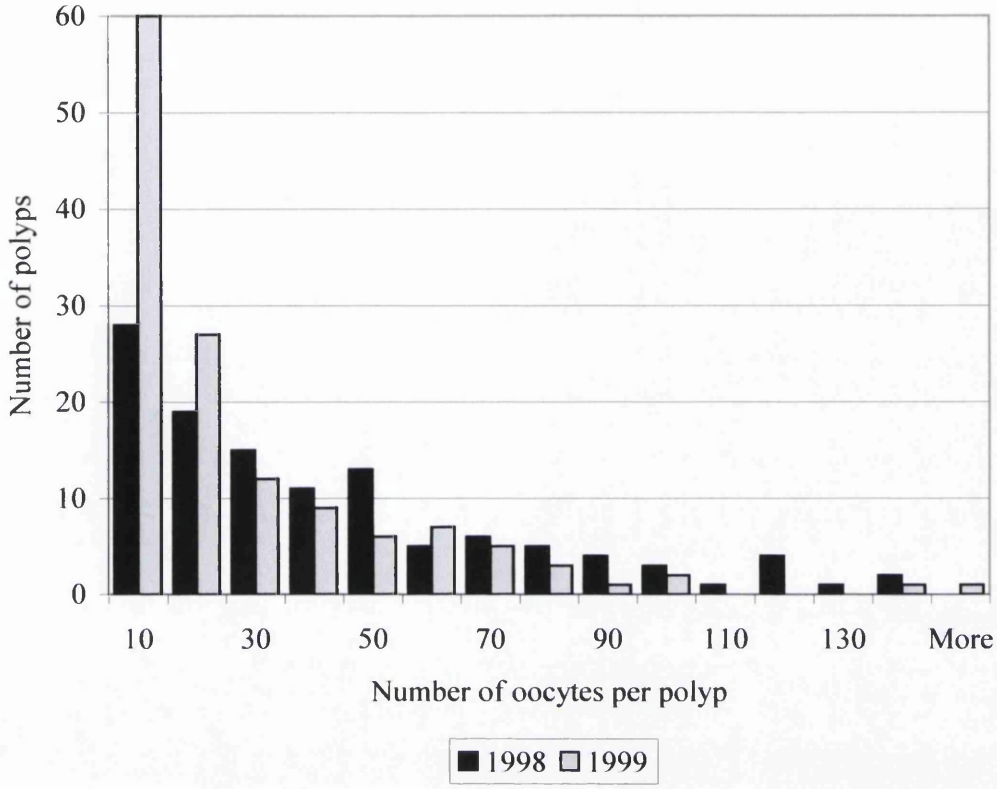
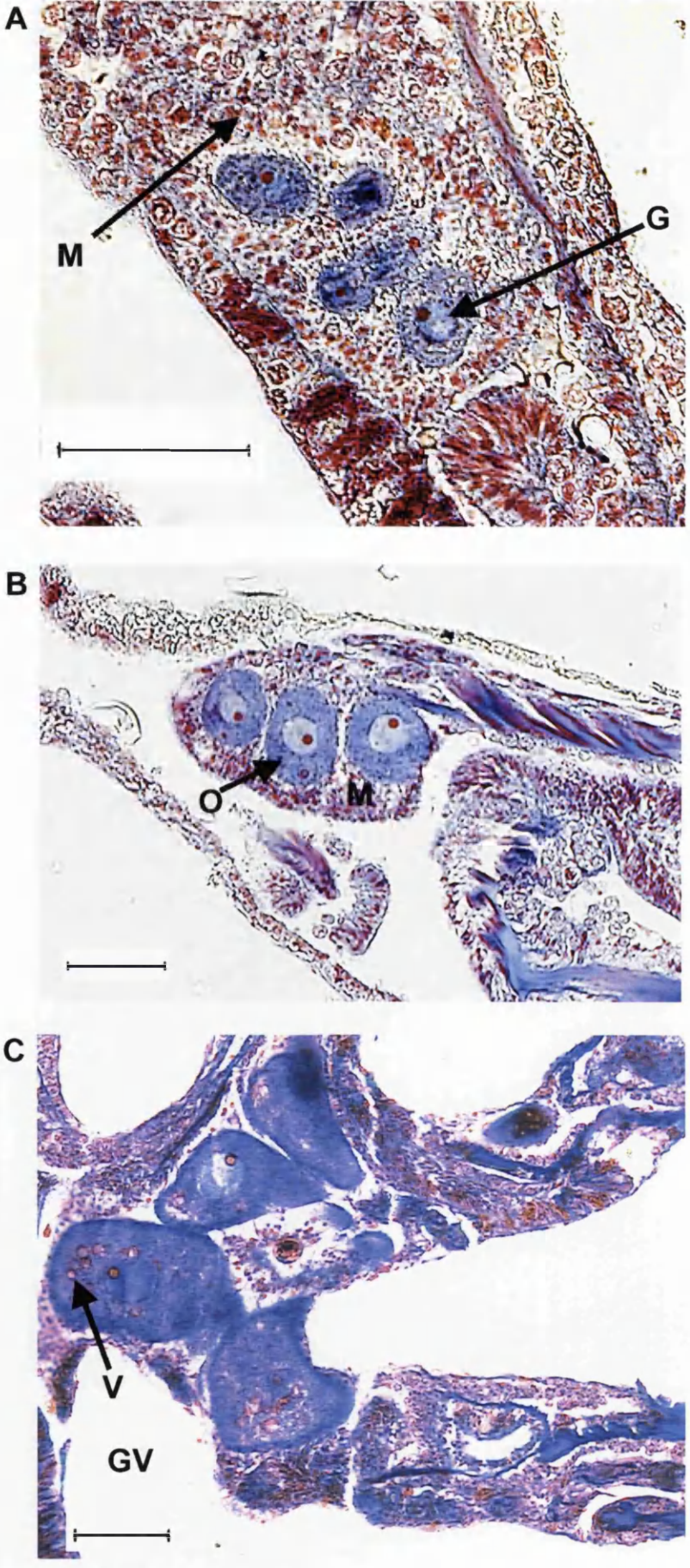


Figure 6.2: Frequency histogram of the number of *Madracis mirabilis* oocytes per polyp. The oocyte counts were combined from all reproductive samples in July, August and September for 1998 and 1999 (total n= 150 polyps in 1998 and 180 polyps in 1999).

Figure 6.3: *Madracis mirabilis* oogenesis. Tissues were fixed in 9% seawater formalin, decalcified with 10% formic acid and stained with Mallory's stain.
All scale bars = 50 μm.

A: Cluster of small oocytes with differentiated germinal vesicle (G) with nucleolus within mesoglea (M) of the mesentery.
B: Oocytes (O) within mesoglea (M) of mesentery.
C: Mature oocytes lying close to the gastrovascular cavity (GV). Oocytes have prominent vacuoles (V) staining red.


A
M
G
B
O
M
C
V
GV

enlarged germinal vesicles (14-18μm, Figure 6.3C). Germinal vesicle growth ceased as the oocytes continued to mature further. The initially round oocytes were commonly oval in cross section when mature and were often seen pushed up towards the gastrovascular cavity from their original position mid-way along the mesenteries (Figure 6.3C). Oocytes were sometimes bell or cone shaped though this can be a consequence of fixation (Chornesky and Peters, 1987). Mature oocytes contained numerous vacuoles, often staining red with the Mallory Stain (V in Figure 6.3D), which would suggest they are lipid droplets (Humason, 1962; Grimstone and Skaer, 1972) that were lost during the histological process (E. Peters, pers. comm.). Maximum oocyte diameter recorded was 161μm.

Small oocytes were present in the earliest samples collected in 1998, at the beginning of July (Figure 6.4). Sampling began earlier in 1999, in May, and there were no oocytes found until June from the Inner Lagoon sampled colonies and until July from the Outer Lagoon colonies (Figure 6.4). The majority of the oocytes by July each year were in the 20-40μm size class with a few larger oocytes reaching >60μm (Figure 6.5 and 6.6). The pattern of increasing mean oocyte diameter through development prior to fertilisation was synchronous between the Inner Lagoon and Outer Lagoon reef sites (Figure 6.4). However, oocyte maturation within and between the colonies was asynchronous as smaller oocytes (<40μm) remained in many colonies throughout the summer (Figure 6.5 and 6.6). In 1998, mean oocyte diameter from the sampled branches was greatest one week before and over the new moon at the end of August (Figure 6.4). The next sample was not collected until September 13th (one week before the September new moon) when oocytes were present only from one of the sites, Crescent B at the Outer Lagoon. All oocytes in this sample were >40μm (Figure 6.5). In 1999, the new moon was earlier in the month and mean oocyte diameter increased over August and peaked early September (Figure 6.5), when there was a greater frequency of larger oocytes (>100μm; Figure 6.6). Oocytes were not found in the subsequent sample 16 days later at the end of September 1999, or in the samples examined in October. Moreover, no small oocytes were detected, indicating that if not shed or fertilised, they were resorbed. There were no observational differences between oocyte development or mean oocyte diameter at the Inner Lagoon and Outer Lagoon reef zones.

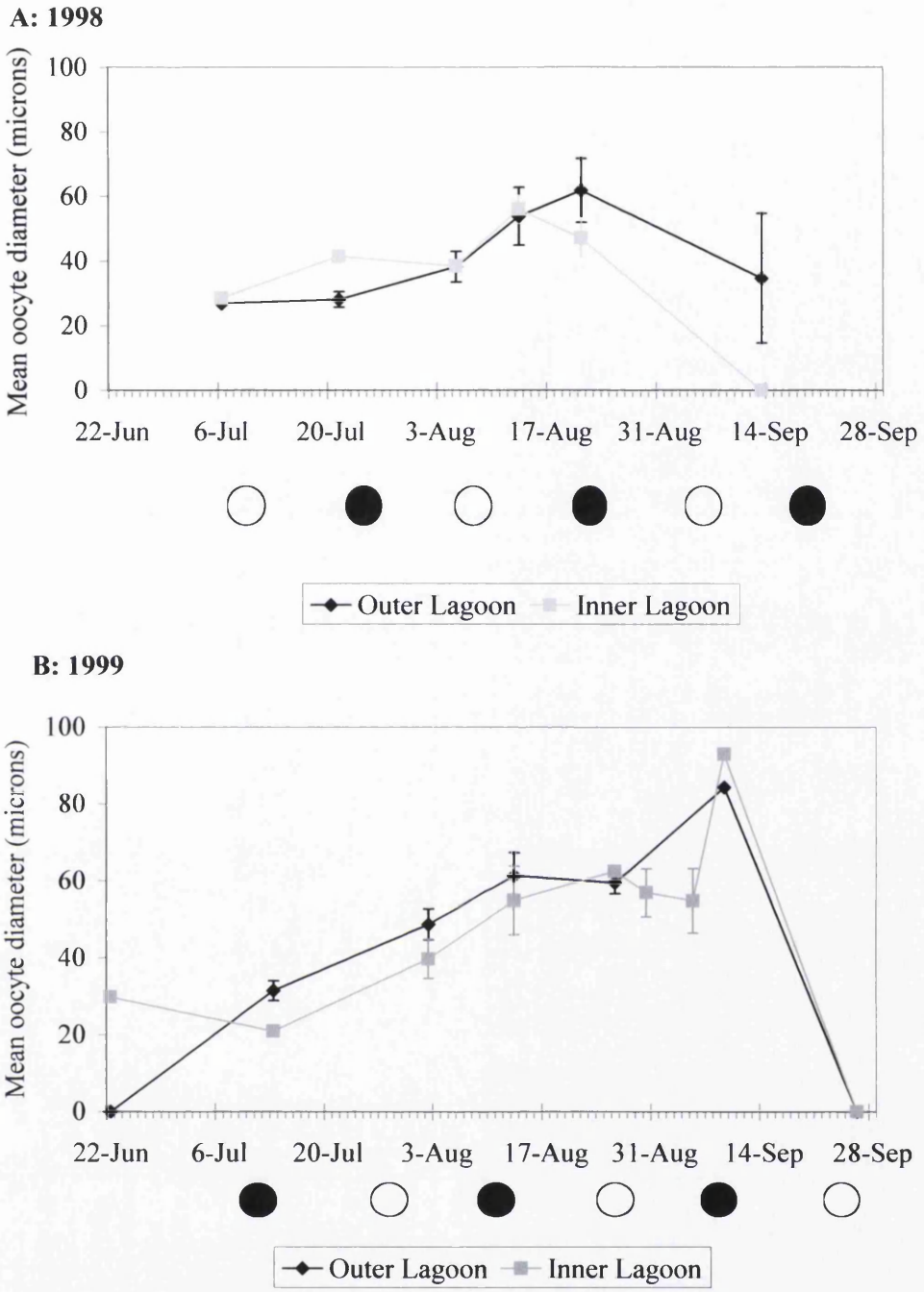


Figure 6.4: *Madracis mirabilis* oocyte growth over the summer of (**A**) 1998 and (**B**) 1999. Oocyte diameter from each reef zone (Outer Lagoon and Inner Lagoon) is the mean of all oocytes in 5 polyps from 2 colonies from each of 2 replicate reefs in 1998 (n=4 colonies per reef zone), and from 3 colonies from each of 2 replicate reefs in 1999 (n=6 colonies per reef zone). Error bars are 1 SE. Samples were also collected before the May 1999 full moon and in October 1999 and no oocytes were present.

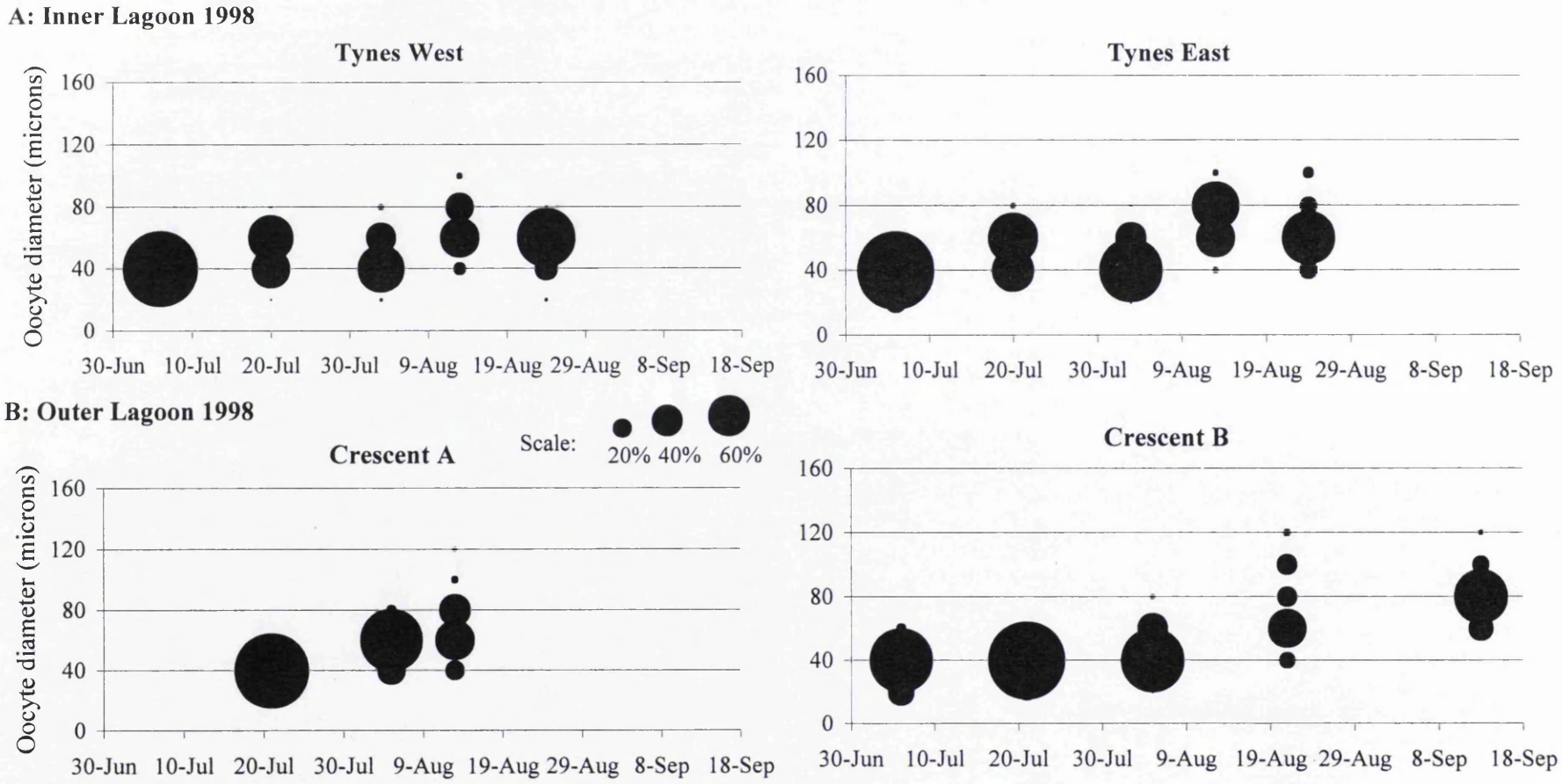


Figure 6.5: Size frequency distributions of *Madracis mirabilis* oocytes over the summer of 1998. Bubble width represents frequency as the percentage of oocytes in each size class. The y axis shows the maximum oocyte diameter of each size class. Oocyte counts are combined from 2 replicate samples at each study site, all oocytes measured within 5 polyps from each sample.

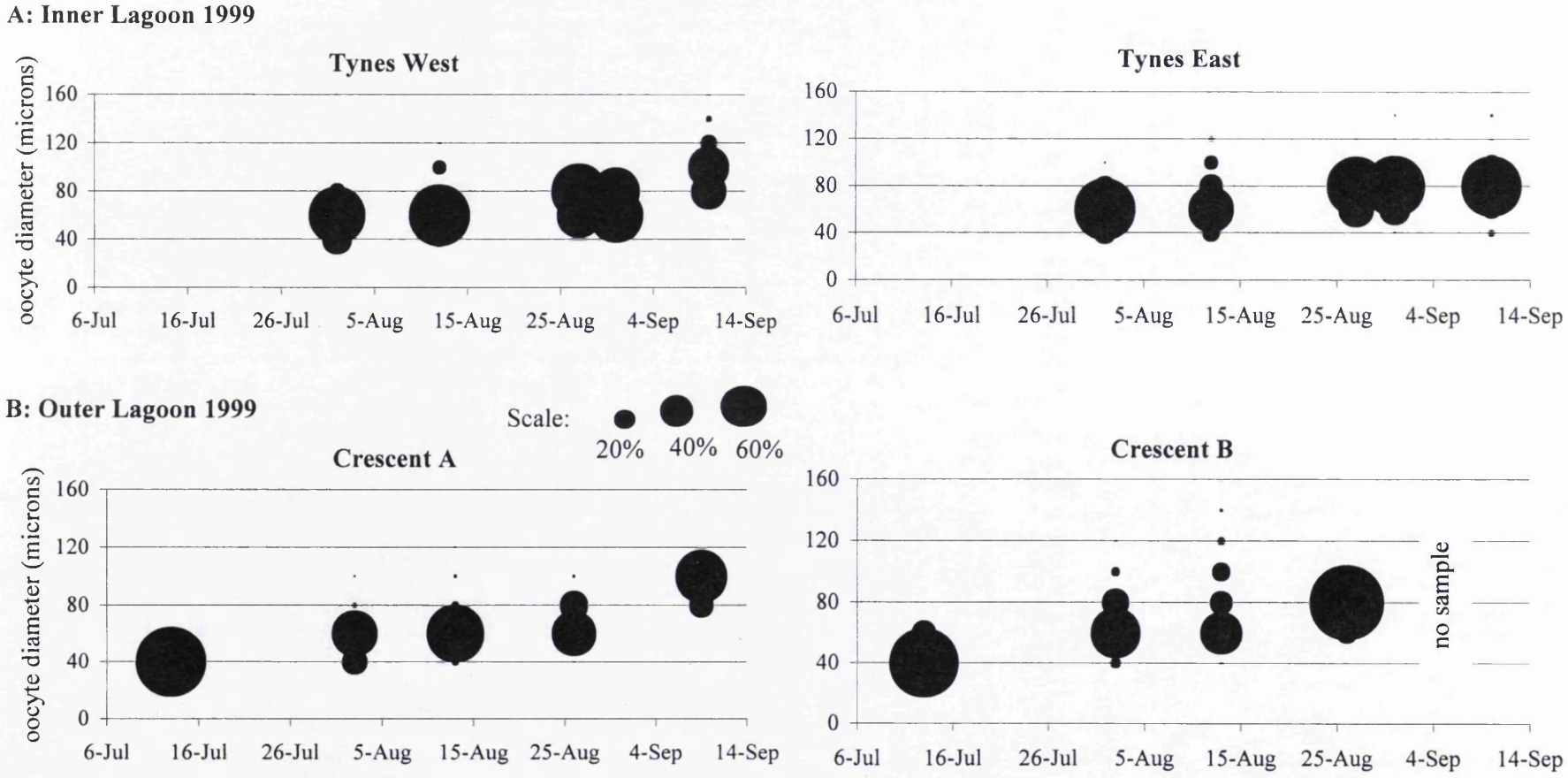


Figure 6.6: Size frequency distributions of *Madracis mirabilis* oocytes over the summer of 1999. Bubble width represents frequency as the percentage of oocytes in each size class. The y axis shows the maximum oocyte diameter of each size class. Oocyte counts are combined from 3 replicate samples at each study site, all oocytes measured within 5 polyps from each sample. The June sample is not included. Small oocytes (15-35microns) were present from the Tynes West site only.

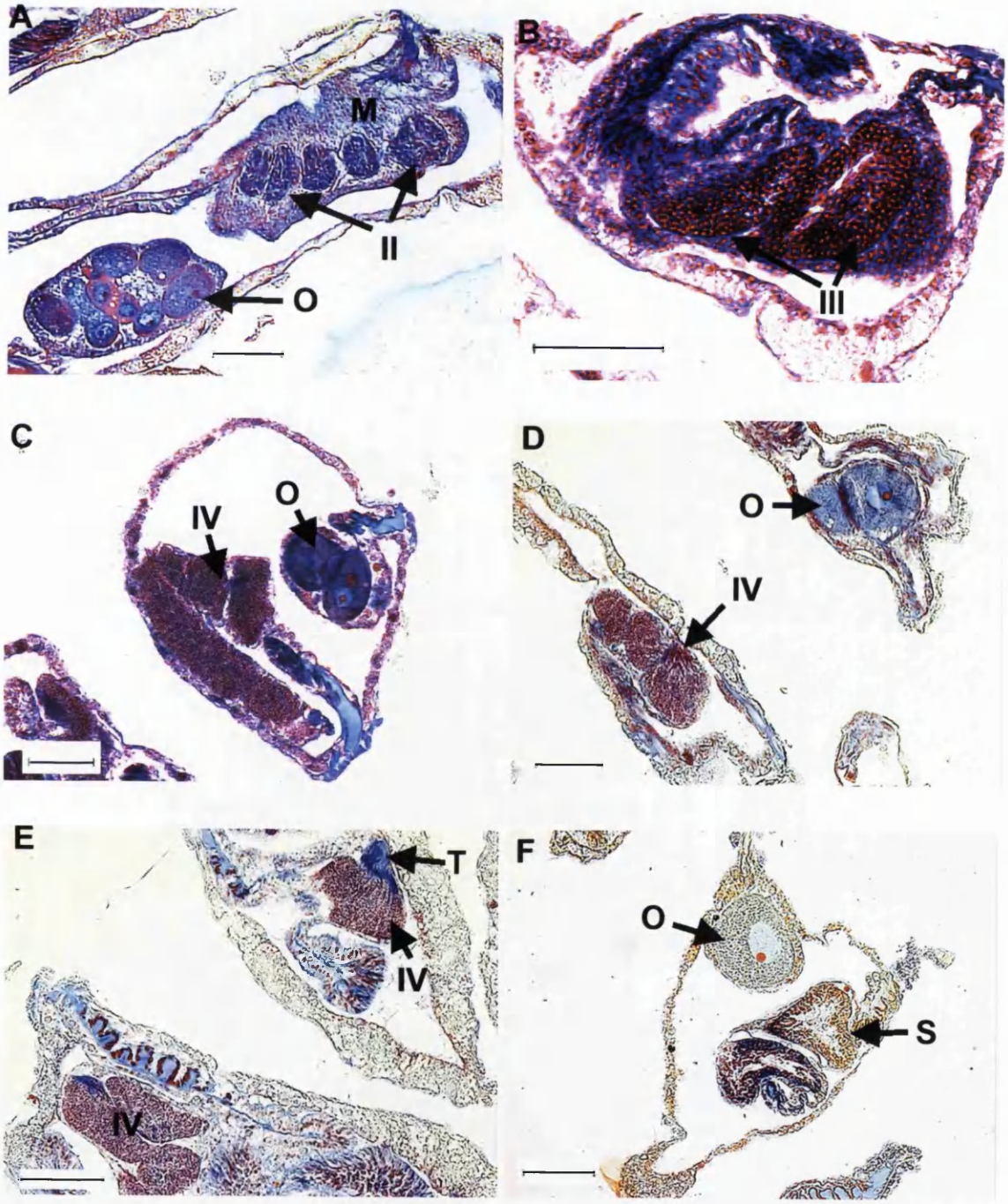
6.4.3 Spermatogenesis

Spermary diameter varied among developmental stages and this is partly a consequence of the random measuring technique, as spermaries are not always measured at their greatest diameter. Spermatogenesis, therefore, was characterised by the developmental stages shown in Table 3.2 (methods) and illustrated by Figure 6.7. Stage 1 of spermatogenesis is the presence of small spermatogonia, which were not found in the sections in the present study. These small spermaries were possibly missed due to the infrequency of the sample dates, the rapid spermary development, and the thickness of the histology sections (8μm). The smallest spermaries observed were 35-45μm in diameter and were stage 2 clusters of primary spermatocytes embedded in thick mesoglea within the mesenteries (II in Figure 6.7A). The development to stage 3 spermaries appeared to be the result of the merging of the smaller stage 2 spermaries as they were less numerous and approximately twice the size. Spermary stage 3 was characterised by a central lumen surrounded by prominent primary spermatocytes of 3-4μm diameter (III in Figure 6.7B, C). The smaller secondary spermatocytes, recognisable by the last mitosis in spermatogenesis, were occasionally seen close to the lumen. They were not easily identifiable, again a consequence of the thickness of the histology sections (8μm). Development to stage 4 spermaries was the result of the division of cells within the spermary with no further increase in overall spermary size. Stage 4 spermaries were full of smaller spermatids of diameter <2μm. In the majority of spermaries, the spermatids had developed to mature spermatozoa ready to be released with prominent tales (IV and T in Figure 6.7D, E).

The stage 2 spermaries were first observed at the beginning of August in 1998 and 1999 (Figure 6.8 and 6.9). Spermaries were not detected in the sampled colonies two weeks prior to this indicating rapid spermary development. A small number of spermaries had developed to stage 3 by the beginning of August in branches from the Inner Lagoon in 1998 (2% of the total number of spermaries) and in branches from the Outer Lagoon in 1999 (13% of the total number of spermaries). One week before the August new moon in 1998 the majority of the spermaries had developed to stage 3 and 4.5% of the spermaries from the Inner Lagoon were matured to stage 4 (Figure 6.8). Over the August 1998 new moon only stage 4 spermaries were present from both reef zones. The

Figure 6.7: *Madracis mirabilis* spermatogenesis. Tissues were fixed in formalin, decalcified with formic acid and stained with Mallory's stain.
All scale bars = 50 μm.

A: Stage 2 spermaries (II) within mesoglea (M) of mesentery. A cluster of small oocytes (O) are present within a separate part of the mesentery.
B: Stage 3 spermaries (III) with central lumen.
C: Mesentery with stage 4 spermaries (IV) and medium sized oocyte (O).
D: Stage 4 spermary (IV) with medium sized oocytes (O) in adjacent mesenteries.
E: Stage 4 spermary (IV) containing spermatozoa with prominent tails (T).
F: Spent spermary (S) with central lumen, and medium sized oocyte (O) in same mesentery.


A
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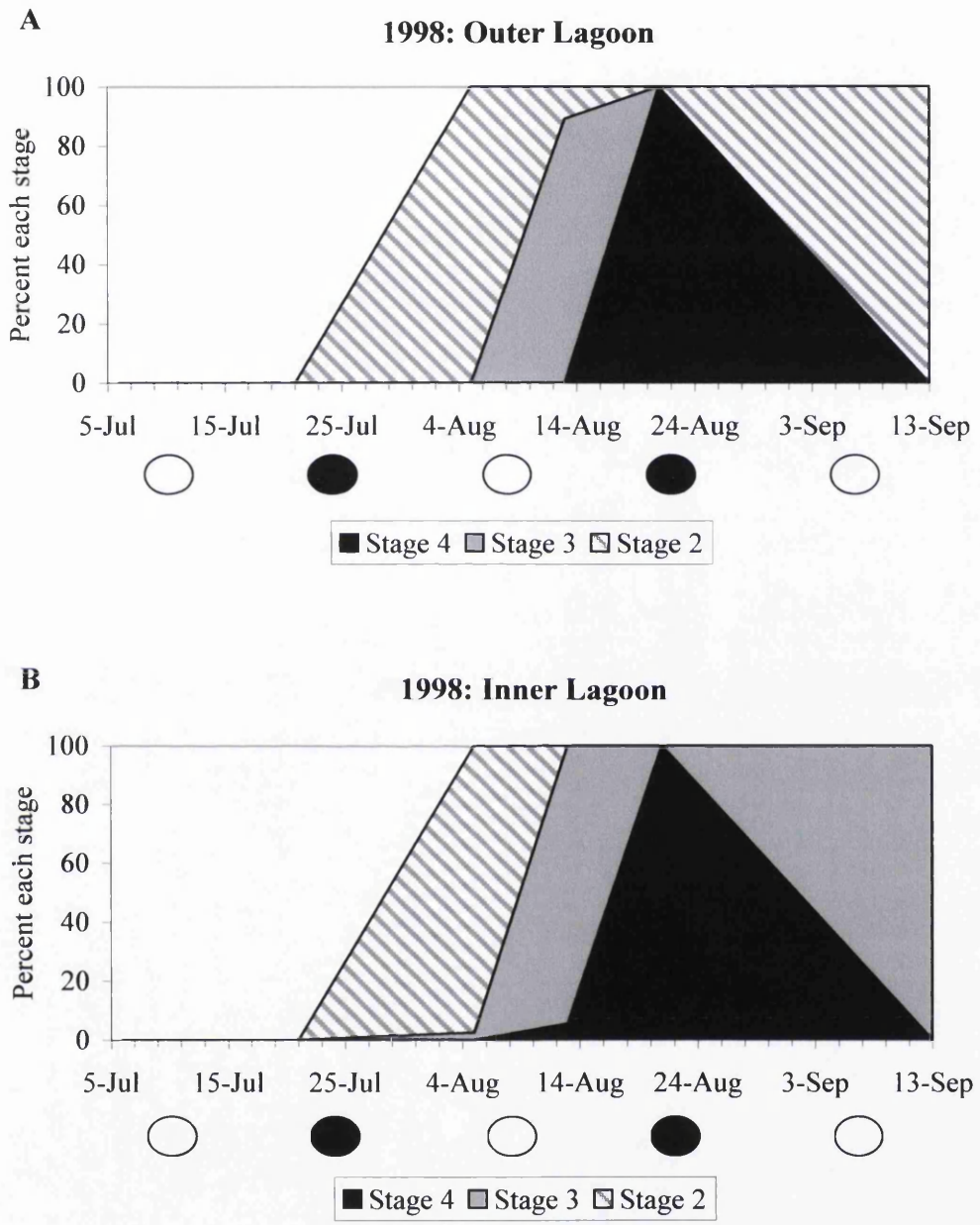


Figure 6.8: *Madracis mirabilis* spermary development for colonies collected from the Outer Lagoon reef zone (A) and Inner Lagoon reef zone (B) in 1998. Occurrence is combined from 2 replicate reefs per zone (n=4 colonies per reef zone on each sample date)

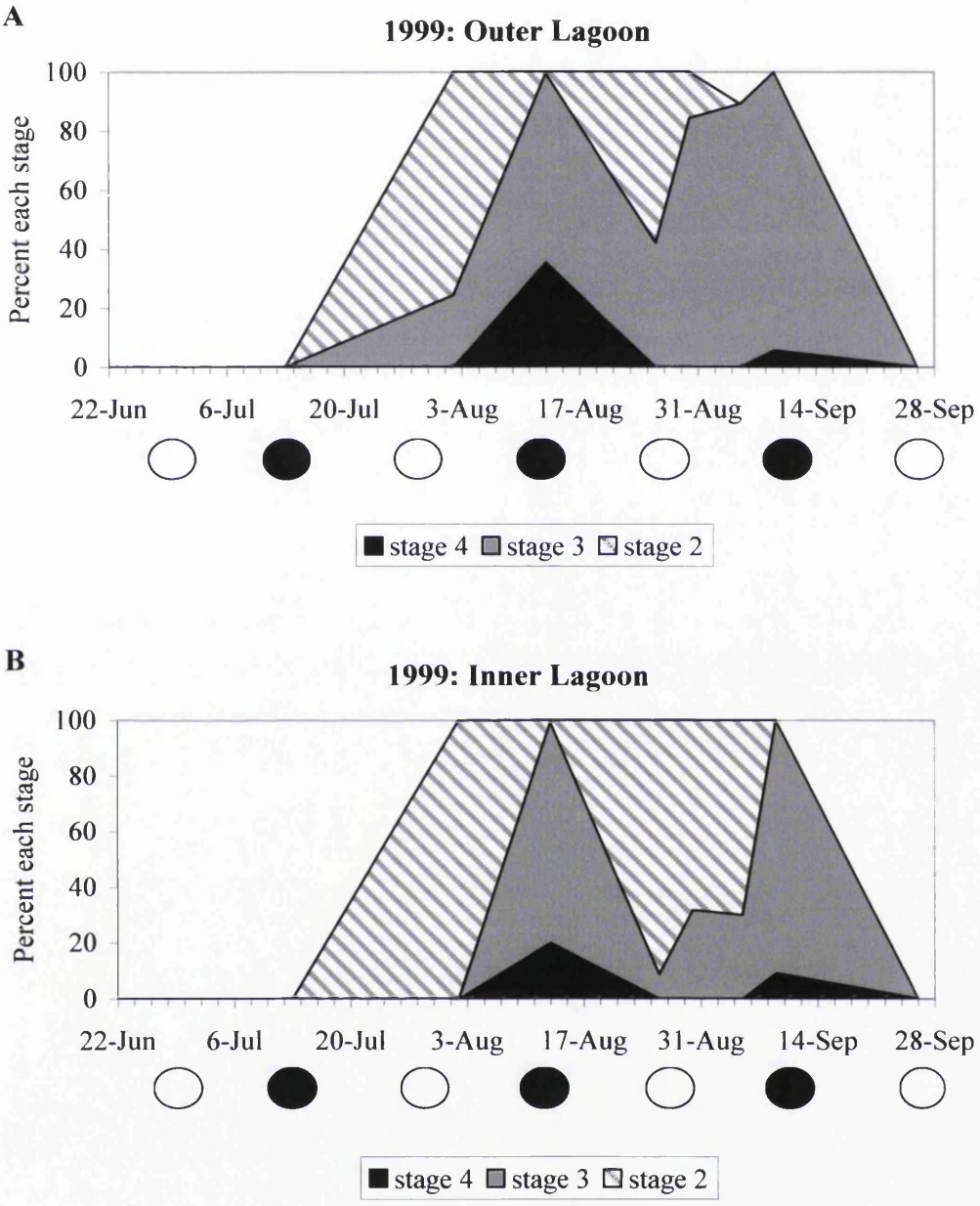


Figure 6.9: *Madracis mirabilis* spermary development for colonies collected from the Outer Lagoon reef zone (A) and Inner Lagoon reef zone (B) in 1999. Occurrence is combined from 2 replicate reefs per zone (n=6 colonies per reef zone on each sample date)

final collection in 1998 was one week before the new moon in September when all stage 4 spermaries had disappeared and stage 2 and 3 spermaries were present indicating the potential growth of a second cohort over September. In 1999, stage 4 spermaries also first appeared around the August new moon from both reef zones, and were absent by the following full moon (Figure 6.9). There was a continued presence of stage 2 and 3 spermaries into September in 1999. A second pulse of growth to stage 4 spermaries was observed around the September 1999 new moon, which occurred earlier in the month than in 1998. No spermaries were found in samples collected from approximately two weeks later, around the full moon at the end of September. Sperm release was not observed in the sections or from live colonies. In two samples from the August new moon in 1998, spent spermaries were seen where mature sperm had been released from the centre of the spermaries with a few sperm heads and tails remaining (Figure 6.7F). Spermatocytes remained around the periphery of the spent spermaries, which possibly developed for a second spawning in September. On some occasions, spent spermaries were seen with medium sized oocytes in the same mesentery (Figure 6.7F), indicating that simultaneous gamete release was not occurring from some polyps.

6.4.4 Relationship of gamete development with seawater temperature

The annual seawater temperature patterns across the physiographic reef zones of the Bermuda platform were discussed in Chapter 2. The seawater temperature range in 1998 and 1999 at the high latitude reefs of Bermuda fell to a winter minimum of 15.5-17°C in February until April, and reached a summer maximum of 29-31°C, dependent on the reef zone. Oocyte maturation, both as mean oocyte diameter and the percentage of oocytes >60μm, follows the increasing seawater temperature in July of 1998 and 1999 (Figure 6.10). Mean oocyte diameter, as well as the percentage of oocytes >60μm, increased at the end of August when the seawater temperature was at its maximum, and into September as seawater temperatures are declining. Mature, stage 4 spermaries were present around the new moon phases in August 1998 (no sample was collected over the September 1998 new moon) and in August and September 1999.

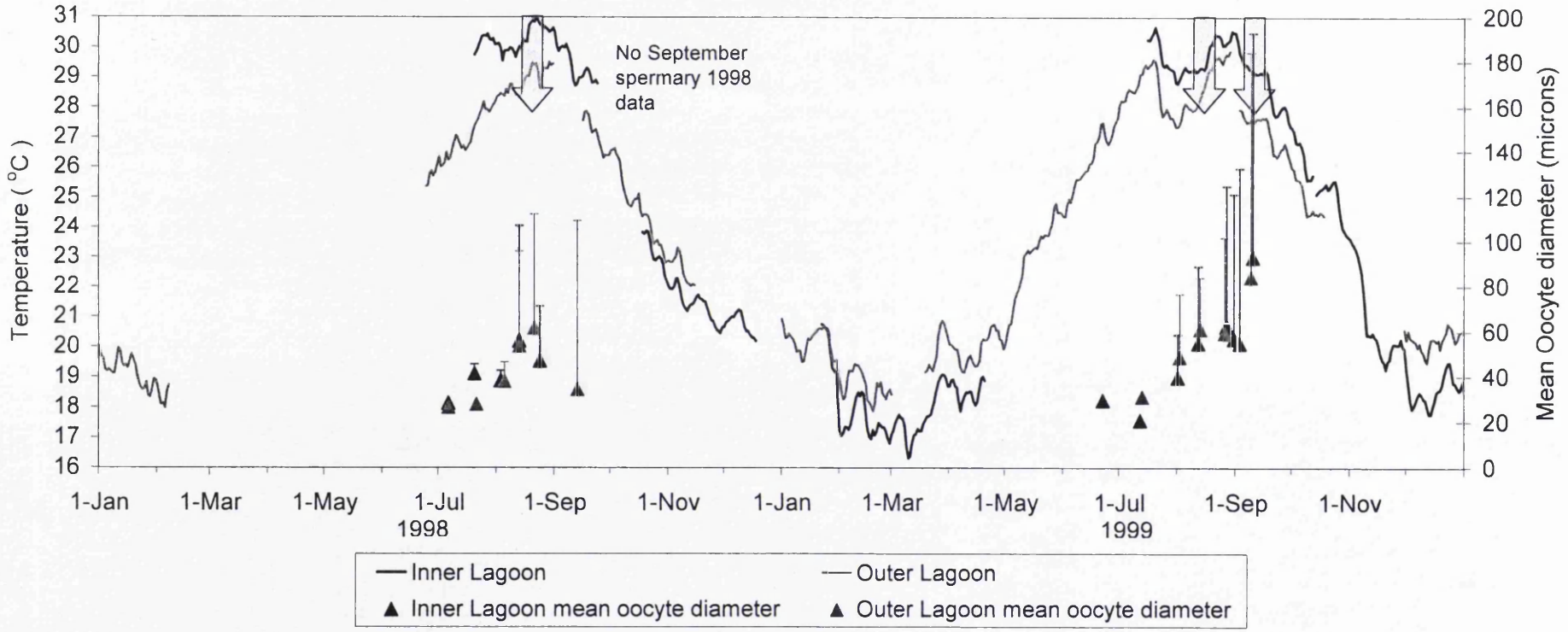


Figure 6.10: Average daily seawater temperature variation over 1998 and 1999 at the Inner Lagoon and Outer Lagoon reef zones. The triangles show *Madracis mirabilis* mean oocyte diameter (right ordinate) and the error bars are percentage of large oocytes >60microns. The arrows indicate when stage 4 spermaries were present (reef zones combined). Gaps in the temperature data are periods when data loggers were not deployed or malfunctioned.

6.4.5 Laboratory monitoring of spawning

Madracis mirabilis branches were collected and held in aquaria for the monitoring of spawning in August and September of 1998 and 2001. Spawning was not directly observed but coral propagules were periodically found in the aquarium seawater. The coral propagules were round, either white or beige and stationary or moving (see Table 6.3A and B, and Figure 6.11A). Their sizes were estimated with an eye piece ocular and were commonly between 200-300μm (min. ~180μm, max. ~530μm). There was no apparent correlation between size, colour or movement of the coral propagules. Over August and September 2001, filtered aquarium seawater was used and the coral propagules captured were similar to those in 1998 (Table 6.3B). An experiment to capture gametes and/or planulae *in situ* was performed using larval traps, which yielded the same variety of coral propagules to the aquarium held colonies (Table 6.3C). In 1998, there was a peak in the abundance of coral propagules present around the August third quarter moon phase. In 2001 abundance also increased around the August third quarter moon, as well as around the August new moon phase and the September new moon (no sample collected around the September third quarter moon).

Slide squash preparations were made of the propagules captured in August and September 2001 and observed under the compound microscope (Figures 6.11). The preparations revealed that the larger coral propagules and a portion of the smaller propagules had a differentiated ectoderm, a thin layer of mesoglea and a homogeneous endoderm (Ec, M and En respectively in Figure 6.11C). Zooxanthellae were present in the endoderm and their chlorophyll pigment glowed red under UV light (Z in Figure 6.11D). Several of the smaller coral propagules had little layer differentiation with no distinct mesoglea, although the endoderm was developed with a few zooxanthellae present (Figure 6.11E and F). The smallest, white propagules that were often found spinning had minimal noticeable layer differentiation, although the ectoderm must be ciliated to cause movement. There were no zooxanthellae present in the pure white propagules. Slide squash preparations of the brown and white stationary propagules ranging from as small as ~180μm and up to ~300μm were similar in appearance to the spinning propagules with varying degrees of tissue differentiation. Mature oocyte diameter was 140-160μm (max 161μm) from the fixed histological sections (section

Table 6.3: Laboratory monitoring of spawning of *Madracis mirabilis* in the summer of 1998 (A) and 2001 (B) and by the use of *in situ* larval traps in 2001 (C).
New moon= lunar day 0, full moon= lunar day 15,
3/4 moon= lunar day 22.

A: 1998 Laboratory monitoring of spawning

		Description of coral propagules				
		White		Beige		
DATE	Lunar Day	Stationary	Moving	Stationary	Moving	TOTAL
6-Aug	14					
8-Aug	16	1				1
11-Aug	19	3				3
14-Aug	22			2		2
15-Aug	23	10	2		5	17
16-Aug	24	6	5	2	4	17
17-Aug	25		4			4
20-Aug	28					0
24-Aug	3	1			3	4
25-Aug	4					0
15-Sep	25					0
17-Sep	27			2	3	5
18-Sep	0					0
20-Sep	1			1	4	5
21-Sep	2					0
	Total	21	11	7	19	

B: 2001 Laboratory monitoring of spawning

		Description of coral propagules				
		White		Beige		
DATE	Lunar Day	Stationary	Moving	Stationary	Moving	Total
10-Aug	20			5		5
15-Aug	25		2		13	15
17-Aug	27		3		1	4
19-Aug	0			1		1
21-Aug	2	5	17		2	24
26-Aug	7					0
15-Sep	27		4	3	5	12
17-Sep	0	2	3		4	9
	Total	7	29	9	25	

C: 2001 *In situ* larval traps

		Description of coral propagules				
		White		Beige		
DATE	Lunar Day	Stationary	Moving	Stationary	Moving	Total
11-Aug	21	16			2	18
13-Aug	23			7	7	14
15-Aug	25	6				6
17-Aug	27	1		9		10
20-Aug	1					0
	Total	23	0	16	9	

Figure 6.11: Coral propagules collected from *Madracis mirabilis* colonies held in aquaria in September 2001. Figures B-F are slide squash preparations photographed under normal and fluorescent light. All scale bars are 100 μm. See text for details.

A: Coral propagules viewed under a dissecting microscope (x20).

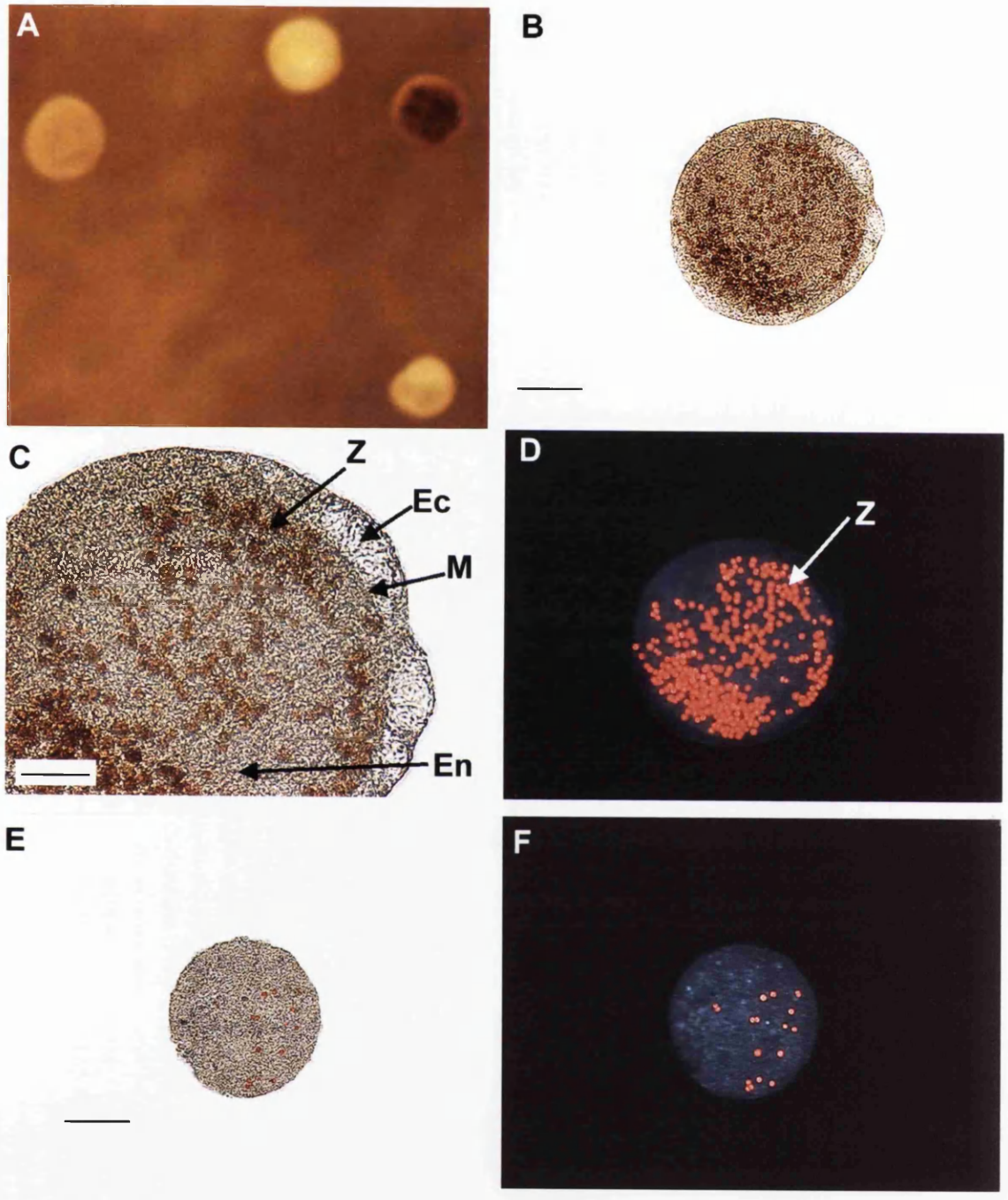
B: Planula (x100), length = 322μm.

C: Same planula as B (x200) showing differentiated ectoderm (Ec) and thin mesoglea (M) surrounding the undifferentiated endoderm material (En) containing zooxanthellae (Z).

D: Same planula as B (x100) under UV light showing zooxanthellae glowing red (Z).

E: Planula (x100), length = 228 μm. Mesogleal layer not distinct.

F: Same planula as E (x100) under UV light.


A
B
C
Z
Ec
M
En
D
Z
E
F

6.4.2). Tissue fixation and histological techniques will cause shrinkage of coral tissue by 20-30% (Harriott 1983a; Table 1 in Ryland, 1997; E. Peters, pers. comm.), therefore estimating the 'true' maximum egg diameter to be 185-210μm. Thus, maximum oocyte diameters were of approximately the same size as the smaller coral propagules collected from the *M. mirabilis* branches.

6.5 Discussion

6.5.1 Gametogenic cycle of *Madracis mirabilis*

Colonies of *Madracis mirabilis* in Bermuda are hermaphroditic at both the polyp and the colony level, in concordance with other members of the genus (Vermeij *et al.*, in review). The male and female gametes of *M. mirabilis* often occur within the same mesentery, developing separately within the mesoglea. Oogenesis was initiated in late June in Bermuda and oocytes were present until the end of September. Spermatogenesis was rapidly completed within one month, with spermaries first detected at the beginning of August. Gametes were not observed from any colonies collected after the end of September concluding the reproductive season of *M. mirabilis* in Bermuda to be over a short period of the summer coinciding with, and just after, maximum seawater temperature. Oocyte development of all *Madracis* species studied in Curaçao in the Southern Caribbean also began in June and spermatogenesis in August, although gametogenesis was extended compared to Bermuda and mature gametes were present from September until October and in some species extended into November (Vermeij *et al.*, in review). The extension of gametogenesis of *M. mirabilis* in Curaçao can be explained by the narrower temperature range, which is ~4.5°C in Curaçao (Vermeij *et al.*, in review) compared to 15°C in Bermuda (Chapter 2). The decreasing temperature range between Bermuda and lower latitudes in the Caribbean is similarly correlated with a lengthening of the reproductive season of the scleractinian *Porites astreoides* and the gorgonian *Pseudoplexaura porosa* (Chapter 5).

Superimposed on the seasonal development of gametes of *M. mirabilis* was a lunar periodicity to the occurrence of mature spermaries over the new moon periods of August and September. Oocyte maturation was asynchronous within the branches with oocytes of all sizes found within each sample, although mean oocyte diameter was at a maximum over the August and September new moon periods. The gametogenic studies on *Madracis* species in Curaçao did not include repetitive samples over each month and so the incidence of lunar periodicity to gamete development could not be determined (Vermeij *et al.*, 2003).

6.5.2 Reproductive mode

Developing embryos or planulae were not found in the histological sections of *Madracis mirabilis* tissues, despite the examination of five polyps from each of 123 branches from different colonies over 18 sample dates in August and September 1998 and 1999. This result parallels that of Vermeij *et al.* (in review), who also found no indication of planula brooding in the study of the six *Madracis* species in Curaçao of the Southern Caribbean. However, coral propagules were collected from *Madracis* colonies held in aquaria and from *in situ* larval traps in the present study, and also by Vermeij and Bak. (2002) and Vermeij *et al.* (2003) in Curaçao. Microscopic examination of the propagules by slide squash preparations revealed a homogeneous endoderm. The formation of endoderm during embryonic growth is by gastrulation of the previous blastula stage, and indicates the end stages of embryogenesis and development to early stage planulae (Mergner, 1971). The blastula stage of development can be distinguished by the presence of dividing ectodermal cells. A study by Babcock and Heyward (1986) on the larval development of several scleractinian species described the formation of endoderm to occur ~24-36hr after spawning. Prior to gastrulation, the embryos of these studied species were hollow blastulae that were first formed 7-10hr after spawning (Babcock and Heyward, 1986). Embryonic development has also been studied in one tropical zoanthid, *Protopalythoa* sp. (Babcock and Ryland, 1990), and similarly occurs via a hollow blastula. A solid yolky mass was formed 17hr after spawning and the endoderm and mesoglea were not differentiated until two days. Embryo development

of the broadcast spawning solitary coral *Astrangia danae*, observed as slide squash preparations, was different in that the cells of the blastula were a solid ball ("stereoblastula", Szmant-Froelich *et al.*, 1980). Gastrulation rapidly occurred 6-8hr after fertilisation and the endoderm was formed 12-15hr after spawning. The blastulae of *Fungia scutaria*, another broadcasting solitary species, are also stereoblastic, the mass of cells visible in slide preparations (Krupp, 1983). Endoderm formation occurred ~18hr after spawning. The slide squashes of *M. mirabilis* propagules never showed a mass of cells or a hollow centre to indicate either a stereoblastual or holoblastula stage. The uniform endoderm observed is characteristic of an early stage planulae. Variation of mesoglea development was seen in the propagules and these are presumably different stages of the post-gastrulation development.

If the planulae were the product of external fertilisation, embryonic development was being completed in 13-16 hours, which was the period of time between isolating the water holding the colonies in the evening and examination for propagules in the morning. This would be a rapid development time, similar to that of *A. danae* (12-15hr, Szmant-Froelich *et al.*, 1980) and *F. scutaria* (18hr, Krupp, 1983), and much faster than the 24-36hr required by the 19 broadcast spawning scleractinians studied by Babcock and Heyward (1986). If internal fertilisation was occurring within the *M. mirabilis* colonies, the duration of brooding must be minimal, maybe just hours to days, as embryos were not found in the histological sections. Furthermore, the planulae collected were of a similar size or only slightly larger than the maximum oocyte diameter recorded from the histological sections (planulae commonly 200-300μm and maximum unfixed oocyte diameter of 210μm, estimated from measurements of fixed material). Externally developed planulae are commonly only slightly larger than oocytes (Krupp, 1983; Babcock and Heyward, 1986), and in *A. danae*, the developed planulae were actually only three-quarters to half the diameter of the oocytes (Szmant-Froelich *et al.*, 1980). In comparison, the planulae of *Porites astreoides* that are brooded for 2-3 weeks inside the 'parent' polyps are densely ciliated and 1-1.5 mm in length, which is much larger than the maximum oocyte diameter of 180μm (Chapter 3). The larger oocytes of *Favia fragum* are up to 250μm in diameter, in contrast to the planulae that are a maximum of 2 mm in length after a three week period of internal brooding (Szmant-Froelich *et al.*, 1985). In order to confirm whether fertilisation is

internal in *M. mirabilis* and the length of the 'brooding' period, if any, histological samples are required over several daily time intervals around the period of maximum gamete presence to look for the varied fertilisation membrane and embryonic stages. Histological sectioning of the coral propagules is also needed at time points during rearing after collection to confirm planula developmental time.

The early release of embryos from a coral colony, instead of internal brooding, has been observed in the branching scleractinian species *Eusmilia fastigiata* (de Graaf *et al.*, 1999). The embryos were seen being released from the colony tentacles and subsequent collection, observation and rearing of the released propagules confirmed that they were fertilised zygotes that were undergoing embryogenesis. The sexuality and gametogenesis of *E. fastigiata* is unknown, so inferences as to the origin of the zygotes (sexual versus asexual and cross fertilisation versus selfing) cannot be made. Internal fertilisation followed by almost immediate release of zygotes is also suspected to occur for the massive scleractinian species *Stephanocoenia intersepta* and *Montastrea cavernosa* (Hagman *et al.*, 1998a). There has been no observation of zygote release from these colonies, although 95% of the released propagules collected directly over female colonies were found to be undergoing stages of cleavage. These massive species are known to be gonochoric (Szmant, 1986; Hagman *et al.*, 1998a) and so the released zygotes are not the product of self-fertilisation. Spawning by male colonies of both species was observed to begin up to one hour before that of the female colonies (Hagman *et al.*, 1998b), further suggesting that internal fertilisation in the female colonies before zygote release is possible. Alternative explanations are either that the male colonies enjoy a very high level of fitness, and fertilisation of all but a few eggs occurs at the female colony surface; or that the embryos have developed parthenogenically, although these theories are not supported by the discussion in Hagman *et al.* (1998b).

The selective advantage of internal fertilisation over broadcast spawning of gametes would be to reduce gamete wastage from the low effectiveness of external fertilisation (Denny and Shibata, 1989). The associated enhanced reproductive success from a brooding strategy is suggested to be the 'driving' force for the selection for internal fertilisation from the believed ancestral mode of broadcast spawning (Ryland and Bishop, 1993; Shlesinger *et al.*, 1998). The "quick-releasing" (sensu Vermeij *et al.*,

2003) or 'pseudo-brooding' strategy may be an evolutionary step towards a brooding mode of reproduction. The key step of internal fertilisation for gonochoric species, such as *Stephanocoenia intersepta* and *Montastrea cavernosa* is clearly an advantage as self fertilisation cannot occur and reproductive success depends on gamete crossing. All *Madracis* species are hermaphroditic (Vermeij *et al.*, in review; this study) and so the internal fertilisation that is suspected to occur in this species may be from cross-fertilisation or the result of selfing, unless the gametes exhibit self-incompatibility. Incidences of self-fertilisation have been shown to occur in hermaphroditic brooders (Brazeau *et al.*, 1998) and broadcasting species (Kojis and Quinn, 1981b; Heyward and Babcock, 1986; Szmant *et al.*, 1997). The obligation for outcrossing between gonochoric colonies is therefore not necessarily a selective force for internal fertilisation in *Madracis* species.

The selection for reproductive mode that may be operating for *Madracis* species is the energetic and mechanical restraints to the colony. The brooding of advanced stage planulae within a coral polyp is energy and space restrictive and there is often a trade-off between size and the number of planulae produced (Harrison and Wallace, 1990). Fecundity of brooding species to be lower than broadcasting species, with smaller and fewer eggs produced. A large amount of energy is invested into the fewer eggs by brooding to late-stage planulae. Fecundity, however, will be dependent on polyp size and some brooding species with relatively large polyps, such as *Favia fragum*, have similar oocyte densities to broadcasting species (see Table 2, Szmant, 1986). The fitness advantage of a 'pseudo-brooding' strategy may be to increase oocyte and planulae fecundity, as less space would be required for the smaller size of the brooded planulae. *M. mirabilis* has a similar polyp size to *Porites astreoides* (~1.5 and 1.2-1.6 mm respectively; Veron, 2000). *P. astreoides* is known to have a brooding reproductive mode (Szmant, 1986; Chornesky and Peters, 1987; Soong, 1991; McGuire, 1998; Chapter 3) and so it is interesting to compare fecundity between the two species. In Bermuda, *P. astreoides* maximum oocyte diameter was 135μm with a mean number of 2.9 oocytes polyp^{-1} (Chapter 3). Maximum oocyte size of *M. mirabilis* was larger, at 161μm (all oocyte estimates here quoted as fixed size) with a greater mean fecundity of 22.7 oocytes polyp^{-1} (section 6.4.1 and 6.4.2). Other estimates for *P. astreoides* give a variable oocyte diameter, although fecundity is always lower than that recorded for

M. mirabilis in Bermuda. Chornesky and Peters (1987) in Jamaica recorded oocyte size of *P. astreoides* as 130-170μm and the greatest median fecundity over the sampled months was 2.3 oocytes polyp^{-1}. *P. astreoides* colonies in Panama had a large maximum oocyte diameter of 200μm and a fecundity of 10 oocytes polyp^{-1} (Soong, 1991). In Puerto Rico, maximum oocyte diameter was much smaller than the *M. mirabilis* colonies at 50μm and there was a large number of the smaller oocytes, up to 20 oocytes polyp^{-1} (Szmant, 1986). The reproductive strategy of *M. mirabilis* therefore allows for the production of a greater number of similar sized oocytes compared to that of *P. astreoides*, a brooding species of similar polyp size. Further studies estimating planula numbers of *M. mirabilis* are needed to determine whether the increase in oocyte fecundity relates to an overall increase in planula abundance.

Coral propagules were collected from the *M. mirabilis* colonies over a period of days, which is indicative of internal fertilisation and brooding, as the broadcasting of gametes normally occurs over brief spawning periods to maximise out-crossing and reduce gamete wastage (Harrison and Wallace, 1990). Unsynchronised gamete release would only be possible in colonies that live in close proximity and if there is a sperm collection and storage mechanism, as occurs in some bryozoan species (Ryland and Bishop, 1993). A peculiarity of the coral propagules collected is that they were of various sizes and developmental stages. This implies either unsynchronised fertilisation of gametes or, if internal fertilisation is occurring, variable brooding periods or embryonic developmental time. The coral propagules also had an inconsistent presence of zooxanthellae, which is suggestive of differential infection rates, either vertically during potential brooding or horizontally during external development, although the presence or absence of zooxanthellae was not always correlated with planula size. There was no difference in the propagules collected from the use of unfiltered or filtered seawater in the aquaria and so the variability of size and zooxanthellae observed was not believed to be contamination of the water supply.

The inconsistency of zooxanthellae density and the variable size of planulae collected from *M. mirabilis* colonies are also possibly suggestive of a mixed reproductive mode, as neither spawning nor planulation was observed. Brooded planulae generally contain zooxanthellae, whereas externally developed planulae are azooxanthellate when first

released (Harrison and Wallace, 1990; but for exceptions see Kojis and Quinn, 1981a; Krupp, 1983; Kojis, 1986a; Rinkevich, 1989; Glynn *et al.*, 1991; Kruger and Schleyer, 1998). Colonies of the scleractinian *Goniastrea aspera* in Okinawa, Japan, were observed to spawn gametes followed by the internal fertilisation and brooding of a portion of retained eggs (Sakai, 1997). *Pocillopora damicornis* also broods planulae as well as spawns gametes in Western Australia, although the planulae are believed to be derived asexually (Ward, 1992). The advantage of combining a brooding and broadcasting strategy is the accumulation of the benefits associated with each reproductive mode. Intra-species plasticity in reproductive mode has previously been related to variable energetic costs at the individual, community and ecological scale selecting for either brooding or broadcasting at different geographic locations of distinct environmental conditions (Richmond, 1987; Glynn *et al.*, 1991; Kruger and Schleyer, 1998). Similar explanations can satisfy the occurrence of a mixed reproductive mode within the same population.

The reproductive mode of corals has been related to parameters such as the nature and predictability of the habitat (Loya, 1976; Stimson, 1978; Van Moorsel, 1983; Szmant, 1986), and the need for dispersal versus replenishment of the immediate parent population (Kojis and Quinn, 1981b; Kojis and Quinn, 1982; Krupp, 1983; Szmant, 1986; see review in Chapter 1). However, the patterns relating these trade-offs are complex, as they are species-specific and often variably correlated with several proposed selective forces. A brooding mode of reproduction in scleractinians has commonly been correlated with short dispersal (philopatry) by the release of late-stage planulae that rapidly settle (Szmant-Froelich *et al.*, 1985; Ward, 1992; but see Richmond, 1987). The resultant high recruitment to the parental habitat by a brooding reproductive mode is suggested to be both advantageous when conditions are favourable, thereby ensuring good conditions for the offspring (Szmant, 1986), and also a preferred reproductive mode when the habitat is unstable, to replenish the frequently disturbed population (Loya, 1976; Stimson, 1978; Szmant, 1986).

The inshore patch reef in Bermuda is a favourable environment for *M. mirabilis* and dense aggregations occur. Furthermore, this species has a narrow habitat preference in Bermuda and colonies do not survive at the Rim Reef zone, and maintain only a sporadic distribution among the patch reefs of the Outer Lagoon and along the Terrace

Reef (Appendix 6.1). Retention in the parental habitat would therefore appear favourable to prevent planulae being dispersed to an unsuitable habitat. However, the Inner Lagoon patch reefs in Bermuda are also a dynamic environment with rapid population changes arising from the unpredictable levels of high sedimentation stress and wave action during periodic storms (S.R. Smith, unpub. data). Dispersal away from the parental habitat may therefore also have advantages. Colonies of *M. mirabilis* elsewhere readily propagate by fragmentation (Bak and Criens, 1981; Bruno, 1998; Nagelkerken *et al.*, 2000), and this is similarly of a common occurrence in the inshore environment of Bermuda. *M. mirabilis* colonies may therefore achieve greater dispersal through asexual propagation than sexual reproduction. Benthic populations that do not depend on long-lived larvae for dispersal maintain sexual reproduction to preserve outcrossing and thereby decrease the potential genetic costs of continuous inbreeding (Jackson, 1986).

The reproductive patterns of *M. mirabilis* growing on the Terrace Reef in Bermuda (>20 m) was not studied. These populations are occupying a different habitat in respect to food availability and temperature profile (Chapter 2). However, light conditions are fairly comparable at the inshore, shallow murky waters versus the decreased light attenuation with depth at the Terrace Reef, particularly during the summer period of higher turbidity offshore (Chapter 2). Additionally, many *M. mirabilis* colonies found at shallower depths of the Terrace platform are confined to the lower light levels of overhangs and cave entrances (T. Murdoch, pers. comm.). The Inner Lagoon and Terrace Reef are also similar in being low energy environments, except during periodic storms. An understanding of the forces that may select for a particular reproductive mode requires a study of the reproductive strategy of *M. mirabilis* at the Terrace Reef, as well as more information on the population dynamics of the species in Bermuda. *M. mirabilis* is similarly found in a variety of habitats and depths throughout the Caribbean (Lewis and Snelgrove, 1990; Fenner, 1993; Bruno and Edmunds, 1997; Vermeij, 2002) and a comparable study of reproductive pattern related to environmental and habitat conditions is needed to document any universal trend in reproductive strategy of *M. mirabilis* and the occurrence of habitat and location specificity to the reproductive patterns.

6.5.3 Geographic variation in planulae characteristics

The presence of zooxanthellae in the planulae collected from *Madracis* in Curaçao was consistent within a species, although it varied between members of the genus (Vermeij *et al.*, 2003). The planulae of *M. mirabilis* and *M. senaria* always contained zooxanthellae, whereas the planulae collected from colonies of *M. decactis* and *M. pharensis* were azooxanthellate. In contrast, the planulae collected from *M. mirabilis* colonies in Bermuda had a variable presence of zooxanthellae. Furthermore, when zooxanthellae were present in the planulae collected from the Bermuda colonies, they were distributed throughout the endoderm. The zooxanthellae of the planulae of *M. mirabilis* in Curaçao were not evenly distributed and concentrated in a brown ring around the oral end (Vermeij *et al.*, 2003). The planulae of *M. mirabilis* in Bermuda and in Curaçao also differ in lunar periodicity to release. The frequency of planulae collected from the colonies in Bermuda appeared to be greater over the third quarter and the new moon periods of the lunar cycle. In contrast, the abundance of *M. mirabilis* planulae in Curaçao did not show any relation to the lunar cycle, a trait shared with the congeneric species *M. decactis* and *M. pharensis* (Vermeij *et al.*, 2003). *M. senaria* in Curaçao was the only species that exhibited periodic planulae release over the third quarter moon phase. Differences in reproductive strategies and the intensity, type and timing of spawning are distinguishing factors separating closely related coral species (Van Moorsel, 1983; Babcock, 1984; Vernon, 1995; Vermeij *et al.*,. 2003; Vermeij *et al.*, in review). The *M. mirabilis* studied in Bermuda exhibits different reproductive characteristics to this species in the Southern Caribbean. A further ecological and morphological study combined with genetic analysis is required to determine whether *M. mirabilis* in Bermuda is a species different from apparent conspecifics in Curaçao.

6.6 Summary

Colonies of *Madracis mirabilis* are hermaphroditic with mature oocytes and spermaries developing over maximum annual seawater temperatures during August and September

in Bermuda. There was lunar periodicity to spermatogenesis with mature spermaries present over the new moon period. Oocyte development was asynchronous, although mean oocyte diameter was also maximal over the new moon. Early stage planulae were collected from the *M. mirabilis* colonies, and they varied in size as well as the presence and density of zooxanthellae. The reproductive mode of *M. mirabilis* is suspected to be either unsynchronised broadcast spawning followed by rapid external embryonic development or, more likely, a 'pseudo-brooding' strategy of internal fertilisation followed by a short and variable brooding period of just hours to days. It is also possible that the species may be adopting a mixed reproductive strategy of both the brooding and broadcasting of gametes. The selective potential of adopting a modified or mixed reproductive strategy, as occurs in *M. mirabilis*, is to encompass the advantages associated with each reproductive mode. This is particularly beneficial when there are varied morphological, physiological or environmental parameters influencing the species and habitat.

The reproductive characteristics of *M. mirabilis* vary geographically. The abundance of planulae collected from the colonies in Bermuda was weakly correlated to the lunar cycle, whereas planula release from conspecific colonies in Curaçao of the southern Caribbean lacked lunar periodicity. The consistency of zooxanthellae infection of the planulae collected from *M. mirabilis* colonies from Bermuda and Curaçao also differed, as well as the distribution of zooxanthellae in the planulae. Further studies are required to determine whether *M. mirabilis* in Bermuda is different or conspecific with populations in the Caribbean.

Chapter 7: General Discussion

This thesis provides new documentation on the reproductive cycle of the scleractinians *Porites astreoides* and *Madracis mirabilis*, and the gorgonian *Pseudoplexaura porosa* in Bermuda. In the present chapter, this information is combined with an up to date review of the reproductive patterns of Caribbean scleractinian (Table 7.1) and gorgonian species (Table 7.2). The small area of the Caribbean relative to the Pacific basin has a impoverished coral reef fauna of approximately 72 scleractinian (Sterrer, 1986) and 50 gorgonian (Cairns, 1977) reef-dwelling species. A previous review documenting the reproduction of Caribbean scleractinians is by Szmant (1986), and Caribbean species are also included in the general review by Richmond and Hunter (1990). Reproductive information was available for only 19 species from these reviews. The timing and mode of reproduction is now known for 34 scleractinian species (though the sexuality of six of these is still unknown, Table 7.1). A review of the current research on gorgonian reproductive ecology is presented in Chapter 1. Reproductive information on 11 Caribbean species has been summarised in Table 7.2 (the timing of spawning for three of these species is unknown). World-wide, the reproductive pattern is currently unspecified for over 1100 (approximately 79%) scleractinian (P. Harrison, pers. comm.), and the majority (over 99%) of gorgonians. These statistics show that, despite the surge of interest in coral reproductive biology over the last 20 years, the current state of knowledge remains highly deficient. Furthermore, coral species are plastic in their reproductive characteristics and recent research has described within-species variations in the timing and mode of reproduction between geographically separate areas, and even within the same population (reviewed in Chapters 1 and 6).

Table 7.1: Summary of the known sexual patterns and timing of the reproductive season of Caribbean scleractinian species. Size refers to the colony (not necessarily the polyps). First letter is colony size: L, large; M, medium; S, small; second letter is colony shape: D, dome; B, branching; and P, plate. The timing of the reproductive season is inferred from the presence of gravid colonies and/or observations of spawning/ planula release. Lat. is the approximate latitude North of the equator (unless otherwise specified).

*spawning only monitored in August.

Species	Size	Timing	Location	Lat	Ref
Hermaphroditic spawners					
Montastrea 'annularis' (possible species complex; see Weil and Knowlton, 1994)	L,D	Aug/Sep	Panama	9°	4
		Sep/Oct	Bonaire	12°	10
		Sep/Oct	Curaçao	12°	18
		Aug/Sep	Puerto Rico	18°	2
		Aug	Mexico	27°	17
		Jul/Aug	Bermuda	32°	11
Montastraea annularis	L,D	Aug*	Puerto Rico	18°	3
		Aug	Mexico	27°	9
Montastraea faveolata	L,D	Aug*	Puerto Rico	18°	3
		Aug	Mexico	27°	9
Montastraea franksi	L,D	Aug	Mexico	27°	9
Diploria strigosa	L,D	Aug/Sep	Panama	9°	4
		Aug	Bonaire	12°	10
		Aug	Puerto Rico	18°	2
		Aug*	Puerto Rico	18°	3
		Aug	Mexico	27°	9,17
		Aug/Sep	Bermuda	32°	11
Diploria clivosa	L,D	Aug/Sep	Panama	9°	4
		Oct	Curaçao	12°	18
Diploria labyrinthiformis	L,D	Aug	Bonaire	12°	10
		Jul	Bermuda	32°	11
Acropora palmata	L,B	Aug/Sep	Panama	9°	4
		Aug	Curaçao	12°	18
		Aug	Bonaire	12°	10
		Aug	Puerto Rico	18°	2

Acropora cervicornis	L,B	Aug/Sep	Panama	9°	4
		Aug	Bonaire	12°	10
		Aug	Puerto Rico	18°	2
		Aug*	Puerto Rico	18°	3

Hermaphroditic brooders

Favia fragum	S,D	Year-round	Panama	9°	4
		Year round	Puerto Rico	18°	2,12
		Feb-Sep	Bermuda	32°	20
Porites astreoides	S,D	Year-round	Panama	9°	4
(mixed sexuality)		Year-round	Jamaica	18°	13
		Jan-Sep	Puerto Rico	18°	2
		April/May-Sep	Florida	25°	14
		July-Aug/Sep	Bermuda	32°	Ch. 3
Agaricia agaricites	M,D	Spring-Summer	Curaçao	12°	15
Agaricia humilis	M,D	Year-round	Curaçao	12°	15
Manicinia areolata	M,D	June-July	Panama	9°	16
Mycetophyllia ferox	M,D	Jan-March	Puerto Rico	18°	2
Scolymia wellsi (referred to as *S. cubensis* in the N. Atlantic)	S solitary	10-11 months	Brazil	17° S	22

Hermaphroditic pseudo-brooders

Madracis mirabilis, M. senaria, M. pharensis, M. decactis	S,B	Sep-Nov	Curaçao	12°	5
Madracis mirabilis	S,B	Aug- Sep	Bermuda	32°	Ch. 6

Gonochoric spawners

Siderastrea siderea	L,D	Aug-Sep	Panama	9°	4
		July-Sep	Puerto Rico	18°	2
Dendrogyra cylindrus	L,D	Aug	Puerto Rico	18°	2
Dichocoenia stokesi	S,D	Aug-Sep/Oct	Florida	25°	6
Oculina varicosa	S,B	Aug-Sep	Florida	25°	8

Gonochoric brooders

Siderastrea radians	S,D	Year-round	Panama	9°	4
		Year-round?	Puerto Rico	18°	2
		May-Sep	Bermuda	32°	20
Porites porites	S,B	Nov-Feb	Barbados	13°	7
Porites furcata	S,B	Year-round	Panama	9°	4

Gonochoric pseudo-brooders

Montastrea cavernosa	L,D	Aug/Sep	Panama	9°	4
		Aug-Sep/Oct	Columbia	11°	19
		Aug-Oct	Bonaire	12°	10
		Sep/Oct	Curaçao	12°	18
		Aug	Puerto Rico	18°	2
		Aug*	Puerto Rico	18°	3
		Aug	Mexico	27°	9,17
		Aug	Bermuda	32°	11
Stephanocoenia intersepta	L,D	Sep	Bonaire	12°	10
		Aug	Mexico	27°	9

Spawners of unknown sexuality

Colpophyllia natans	L,D	Aug*	Puerto Rico	18°	3
		Aug	Mexico	27°	9

Brooders of unknown sexuality

Agaricia fragilis	S,P	Summer	Florida	25°	1,21
		May-Sep	Bermuda	32°	20
Agaricia crassa (sps. status doubtful)	S,P	Spring	Florida	25°	1,21
Meandrina meandrites	M,D	July/Aug	Florida	25°	1,21
Isophyllia sp.	S,D	Spring	Jamaica	28°	1,21

Pseudo-brooders of unknown sexuality

Eusmilia fastigiata	S,B	Sep/Oct	Bonaire	12°	10

Reference source: 1, extract from Richmond and Hunter (1990); 2, Szmant (1986); 3, Steiner (1995); 4, Soong (1991); 5, Vermeij *et al.* (in review); 6, Hoke *et al.* (2002); 7, Tomascik and Sander (1987); 8, Brooke (2002); 9, Hagman *et al.* (1998b); 10, de Graaf *et al.* (1999); 11, Wyers *et al.* (1991); 12, Szmant-Froelich

et al., 1985; 13, Chornesky and Peters, (1987); 14, McGuire (1998); 15, Van Moorsel (1983); 16, Johnson, 1992; 17, Gittings *et al.* (1992); 18, Van Veghal (1993); 19, Acosta and Zea, 1997; 20, Kuffner and Smith, unpub. data.; 21, extract from Fadlallah (1983); 22, Pires, 2000.

Table 7.2: Summary of the known sexual patterns and timing of the reproductive season of Caribbean gorgonian species. The timing of the reproductive season is inferred from the presence of gravid colonies and/or observations of spawning. Lat. is the approximate latitude north of the equator.

Species	Timing	Location	Lat	Ref
Gonochoric spawners				
Pseudoplexaura porosa	June-Sep	Panama	9°	1
	July-Aug/ Aug-Oct	Bermuda	32°	Ch. 4
Plexaura kuna	May-Sep	Panama	9°	2
Plexaura flexuosa	May/June-Sep	Panama	9°	3
Plexaura homomalla	June-August	Florida	25°	4
Plexaurella sp.	Summer	Florida	25°	7
Pseudopterogorgia americana	??	Panama	25°	7
Pseudopterogorgia acerosa	??	Bahamas	25°	7
Leptogorgia virgulata	??			5
External brooding				
Briareum asbestinum	May-July	Panama	9°	6
Pseudopterogorgia elisabethae	Nov-Dec	Bahamas	25°	8
Pseudopterogorgia bipinnata	Dec	Jamaica	18°	9
	Jan-Feb	Bahamas	25°	7

1, Kapela and Lasker (1999); 2, Brazeau and Lasker (1989, as *Plexaura* A); 3, Beiring and Lasker (2000); 4, Bayer (1974); 5, Adams (1980) cited from Gotelli (1988); 6,

Brazeau and Lasker (1990); 7, H. Lasker, pers. comm.; 8, Gutiérrez-Rodriguez and Lasker (in review); 9, Kinzie (1970).

The three coral species studied in this thesis varied in reproductive mode and sexuality. The scleractinian *Porites astreoides* has a brooding reproductive mode with a mixed sexuality (Chapter 3); the gorgonian *Pseudoplexaura porosa* exhibits gonochorism with broadcasting (Chapter 4), and the scleractinian *Madracis mirabilis* has hermaphroditic colonies with a proposed 'pseudo-brooding' reproductive mode (Chapter 6). This research highlights the simplicity of the original depiction that corals adopt either a broadcasting or a brooding reproductive mode, and are either gonochoric or hermaphroditic (Szmant, 1986). Intermediate modes of reproductive pattern occur both with reproductive mode ('pseudo-brooding'; Chapter 6) and also with sexuality (mixed sexuality of the population; Chapter 3).

Szmant (1986) correlated the reproductive mode of Caribbean scleractinian corals with colony size: hermaphroditic and gonochoric broadcasting species having a large colony size, and all brooding species a small colony size. The Caribbean species documented as hermaphroditic spawners since Szmant's (1986) model adhere to this relationship, and have large colonies, either dome shaped or branching (Table 7.1). However, two exceptions have arisen among gonochoric broadcasting species: *Dichocoenia stokesi* and *Oculina varicosa* were documented to be spawners, despite their small colony size (Table 7.1). The selective pressures leading to the adaptation and evolution of reproductive patterns in coral species are diverse, including life-history traits, habitat, the requirement for dispersal versus retention of propagules, and variation in recruitment rate (reviewed in Chapter 1). It is not surprising, therefore, that exceptions occur to the original model, as Szmant (1986) indeed predicted, and it is likely that future research will describe additional modifications to coral reproductive patterns.

All recently described hermaphroditic and gonochoric brooding species are indeed of a small colony size, although the table does include the crude category of 'medium' sized to incorporate the wide variation in the colony size of brooding species. The proposed 'pseudo-brooding' reproductive mode occurs in species of both small and large colony size, and branching or dome morphology. The variation in colony size, habitat and life history trait of the coral species that have adopted this reproductive strategy implies that

there are mixed selective pressures, as discussed in Chapter 6. The lack of knowledge of the sexual reproduction of many gorgonian species encourages caution in the assignment of 'typical' reproductive patterns. However, all gorgonian species studied to date in the Caribbean are gonochoric and this is combined with a broadcasting or external brooding reproductive mode (Table 7.2). Internal brooding is known to occur in three species outside of the Caribbean and is suspected from a further two species (Chapter 1).

This study of three coral species in the sub-tropical environment of Bermuda documented the influence that the local strong seasonal temperature profile had on their reproductive cycle. The duration of the breeding season of *Porites astreoides*, *Pseudoplexaura porosa* and *Madracis mirabilis* in Bermuda was shorter than that of conspecifics in the Caribbean, and this can be associated with the decrease in annual temperature range towards the equator (Chapter 5 for *Po. astreoides* and *Ps. porosa*; and Chapter 6 for *M. mirabilis*). A similar relationship between latitudinal variation in sea water temperature range and the duration of planulation occurs for some Pacific brooding species (see Chapter 1), and may be more prevalent in the Caribbean, the trends possibly hidden as reproductive information for a species is often restricted to single locations (Table 7.1). However, unpublished data from Bermuda shows that the extreme temperature range at this high latitude reef does not restrict the reproductive season of all brooding species. For example, *Favia fragum* has been observed to release small numbers of planulae as early as February in Bermuda, when water temperature and photoperiod are close to minimum, and *Agaricia fragilis* and *Siderastrea radians* began releasing planulae in May (Kuffner and Smith, unpub. data; the incomplete data does not include all months). The reproductive seasons of many other Caribbean brooding corals are similarly not synchronised within a geographic location (Table 7.1). For example, in Puerto Rico, *Mycetophyllia ferox* releases planulae from January until March, whereas *S. radians* and *F. fragum* planulate year round. Several brooding species studied in Panama have a continuous breeding season (documented for *S. radians, F. fragum,* and *Porites furcata*). In contrast, spawning of *Manicinia areolata* in Panama is restricted to June and July. Thus, it is clear that the parameters determining successful reproductive strategies in corals are species and location specific, precluding the formation of any single predictive model. More research is needed on the cues that select for coral reproductive strategy across geographic

locations, in addition to extending knowledge to the extensive number of species for which the reproduction is not known, or is poorly understood.

The broadcasting gorgonian *Pseudoplexaura porosa* spawned for two months in Bermuda, with a weak spawning over a third month for some colonies (Chapter 4). The reproductive season of conspecifics in Panama is slightly longer, with colonies releasing gametes over three to four months (Table 7.2). Other gorgonian spawners in Panama are reproductive for five months over maximum seawater temperatures (*Plexaura kuna* and *Plexaura flexuosa*), whereas at the mid-latitude of Florida, *Plexaura homomalla* spawned over three months (Table 7.2). Thus, the reproductive seasons of the Caribbean gorgonian broadcasting species studied to date lengthens with decreasing latitude. However, relationships cannot be concluded from the small number of species for which reproductive information is known, and further research is required. The three gorgonian species that externally brood planulae have variable reproductive periods. *Pseudopterogorgia elisabethae* and *P. bipinnata* are winter spawners in the Bahamas, and the latter also spawns in the winter in Jamaica. In contrast, *Briareum asbestinum* is reproductive over the summer at the lower latitude reef of Panama.

The reproductive season of scleractinian broadcasting species is fairly homogeneous throughout the Caribbean. There is a greater variation in the spawning times and degree of synchrony among the distributional range of Pacific broadcasting species, which occur over extended latitudes compared to the smaller Caribbean (see review in Chapter 1; Richmond and Hunter, 1990). Spawning in the Caribbean is generally restricted to one or two months at the time of and just after maximum seawater temperature, which occurs in August and September at most locations (Table 7.1). Spawning of some coral species studied from Bonaire and Curaçao is extended into October, in conjunction with the local delayed annual maximum seawater temperature (Table 7.1). The longer period of spawning of some gorgonian broadcasting species over five months at lower latitudes compared to the brief reproductive season of scleractinian broadcasters at the same latitudes may reflect varying energy budgets between the anthozoan orders, or different environmental cues controlling reproduction, as is discussed in Chapter 5. It is typical, however, that the reproductive cycles of scleractinian brooding species are protracted over several more months than scleractinian broadcasters (Fadlallah, 1983a; Harrison and Wallace, 1990; Richmond, 1997). Whereas the extension of favourable

temperatures often causes brooding reproductive cycles to lengthen with decreasing latitude, the energy budget of broadcasting species may still restrain the breeding season to a few months. Thus, whereas the initiation of gamete maturation may be temperature dependent, the duration of the reproductive season may be dependent on the level of energy allocation to reproduction by the colonies.

Corals with short reproductive seasons, such as some species at high latitudes like Bermuda, will potentially have a reduced yearly reproductive effort compared to conspecifics in the lower Caribbean. If the fecundity of the corals that are only reproductively active over a few months is also reduced, overall reproductive effort will be further compromised. *Porites astreoides* has been studied across many locations in the Caribbean (Table 7.1) and the fecundity of this species is variable. Whereas oocyte fecundity in *Po. astreoides* colonies was comparable between Bermuda and conspecifics in Jamaica, colonies in Panama and Puerto Rico were estimated to have a greater oocyte density (although maximum egg size of the colonies in Puerto Rico was approximately two thirds smaller than recorded in Bermuda). Colonies of *Ps. porosa* in Bermuda had similar oocyte fecundities to conspecifics in Panama, although spermary densities in the Bermuda colonies were over a half lower than that estimated for colonies in Panama. Comparable information on the fecundity of *Madracis mirabilis* colonies between Bermuda and the Caribbean is not available (oocyte fecundity of only mature oocytes recorded in Curaçao). At least two of the species studied in Bermuda, therefore, have their overall reproductive effort reduced compared to populations in the lower Caribbean, since their reproductive season is shorter and they have a similar or lower monthly fecundity.

Coral species at high latitudes that have a low yearly reproductive effort are more sensitive in terms of any disruption to the reproductive months compared to species at lower latitudes that have longer reproductive seasons and/or a greater monthly reproductive effort. Furthermore, the environmental conditions at high latitude reefs delineate the extreme of hermatypic coral survival. With any change in global environmental patterns, high latitude reefs are those that will be the most vulnerable to change since tolerance levels are already at their limits. Seawater temperature was correlated to the monthly reproductive effort of *Po. astreoides* and *Ps. porosa* (Chapter 5). This reliance on seawater temperature, both on the scale of months and on the total

duration of the reproductive season, makes these coral populations susceptible to future temperature change. Furthermore, this study showed variation in seawater temperature to have a contrasting effect on the reproductive effort of the species (Chapter 5), which will have implications on the species response to any future seawater temperature change. The positive relationship between *Ps. porosa* reproductive effort and seawater temperature implies that this species will continue to reproduce under increased temperature, whilst may be sensitive to seawater cooling. In contrast, *Po. astreoides* populations in Bermuda would be susceptible to future seawater warming, an increase in temperature causing a decrease in reproductive effort. Considering the documented effect of temperature on the reproductive effort of the study species in this thesis, it can be said that changing global temperatures may have a profound effect on coral species.

Understanding the ecological processes occurring at marginal reef areas provides information on the controlling factors determining reproductive success and therefore future reef development. It was originally suggested that the corals inhabiting sub-tropical areas of great environmental variation would be reproductively inactive, and reef development only continued by seeding from nearby reefs of lower latitude (Johannes *et al.*, 1983; Veron, 1995). Subsequent research has confounded this paradigm with examples of reproductive coral populations at high latitude and marginal reefs (Harriott, 1992; Kenyon, 1992; Babcock *et al.*, 1994; Van Woesik, 1995; Harii *et al.*, 2001). The coral species inhabiting the sub-tropical reefs of Bermuda are also sexually reproductive, although this thesis has shown that fecundity, as well as the timing of reproduction of at least some species is sensitive to even small temperature changes, and therefore the populations are vulnerable to environmental change.

Appendix 3.1: The surface areas of *Porites astreoides* colonies collected to monitor planula release: Testing the data for normality and variance homogeneity

Kolmogorov-Smirnov test for normality (Wilkinson *et al.*, 1992) performed by the statistical package SYSTAT 5.2.

The normality test was first performed on all reproductive colonies. The successful transformation to cause normality was then tested on the other data.
* is a significant result ($P<0.05$) indicating that the data are not normally distributed.

Sample	n	raw data	arc sine	sq root	log
		P value	P value	P value	P value
Reproductive colonies	93	0.001*	0.013*	0.013*	0.145
Non-reproductive	33	0.526	-	-	0.658
Inner Lagoon colonies	27	0.108	-	-	0.698
Outer Lagoon colonies	31	0.566	-	-	0.499
Rim Reef colonies	35	0.034*	-	-	0.420

The log transformed data were then tested for variance homogeneity using Bartlett's test for homogeneity of variances (Sokal and Rohlf, 1995) performed by the statistical package BIOMstat 3.

1. Testing homogeneity of variances between the surface areas of the reproductive and non-reproductive colonies.
Total sample size: 126
Bartlett's test:
$\chi^2 =$ 0.5267, df = 1
$c =$ 1.011352
$\chi^2 c =$ 0.5208, P = 0.4705
The variances are homogeneous ($P>0.05$)

2. Testing for homogeneity of variances between the surface areas of the colonies collected from the three reef zones.

Total sample size: 126

Bartlett's test:

χ^2 = 1.1099, df = 2

c = 1.011061

$\chi^2 c$ = 1.0978, P = 0.5776

The variances are homogeneous (P>0.05)

Appendix: 3.2: ANOVA to examine for differences between the mean surface area of (1) reproductive and non-reproductive *Porites astreoides* colonies and (2) between the surface area of the *P. astreoides* colonies collected from the three reef zones: the Inner Lagoon, Outer Lagoon and Rim Reef.

1. Reproductive and non-reproductive colonies

Sample size: 126

ANOVA Table

Level	SS	df	MS	Fs	P
sample	0.0025	1	0.00253	0.1724	0.6787
Within	1.8197	124	0.01468		

There is no significant difference between the surface areas of the reproductive and non-reproductive colonies ($P>0.05$)

2. Colonies from the different reef zones

Sample size: 126

ANOVA Table

Level	SS	df	MS	Fs	P
sample	0.4379	2	0.21894	19.4528	$4.566\ 10^{-8}$
Within	1.3844	123	0.01126		

There is a significant difference between the surface areas of the colonies collected from the three reef zones ($P<0.001$)

Multiple comparison of means to test for differences between the colonies collected from the three reef zones: the Inner Lagoon, Outer Lagoon and Rim Reef:

The Tukey-Kramer (TK), GT2 and T′ tests were performed.

* indicates P<=0.050 for at least one method.

Comparing sample 3 against:

Sample:	2	1
Diff:	0.0549*	0.1468*
MSD(TK):	0.0528	0.0559
MSD(GT2):	0.0539	0.0570
MSD(T'):	0.0544	0.0603

Comparing sample 2 against:

Sample:	1
Diff:	0.0920*
MSD(TK):	0.0573
MSD(GT2):	0.0584
MSD(T'):	0.0603

Appendix 3.3: The number of planula released per day from *Porites astreoides* colonies monitored in aquaria: testing for normality of the data

Kolmogorov-Smirnov test for normality (Sokal and Rohlf, 1995) performed by the statistical package SYSTAT 5.2.

The normality test was first performed on planula release per day from all colonies. The successful transformation to cause normality was then tested on the other data separated to reef zone.

* is a significant result ($P<0.05$) indicating that the data are not normally distributed.

Sample	n	raw data	arc sine	sq root	log
		P value	P value	P value	P value
All planulae	93	0.000*	0.000*	0.000*	0.119
Inner Lagoon planulae	27	0.000*	-	-	0.499
Outer Lagoon planulae	31	0.000*	-	-	0.929
Rim Reef planulae	35	0.000*	-	-	0.166

Appendix 3.4: The relationship between lunar periodicity and the percentage of *Porites astreoides* colonies releasing planulae, and the number of planula released, from colonies collected at the Inner Lagoon, Outer Lagoon and Rim Reef zones of the Bermuda platform: testing for normality of the data

Kolmogorov-Smirnov test for normality (Sokal and Rohlf, 1995) performed by the statistical package SYSTAT 5.2.

1. The percentage of colonies that released planulae on each day from the Inner Lagoon, Outer Lagoon and Rim Reef (data for each reef zone is percentage of the total number of colonies monitored from July and August 1999 and 2000). N= 21 days of monitoring for planula release (10 days either side of the new moon).

* is a significant result ($P<0.05$), indicating that the data are not normally distributed.

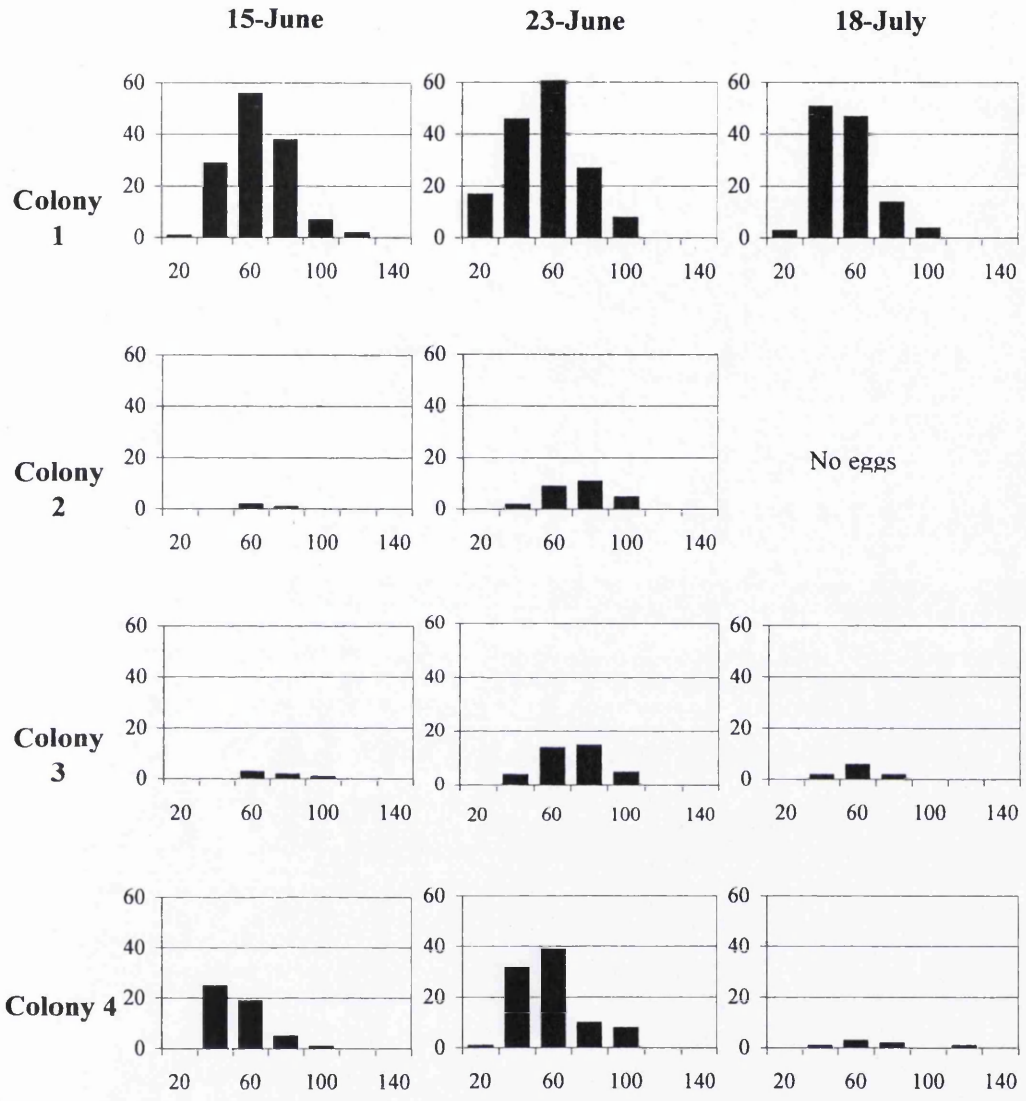
Sample	n	raw data	arc sine	sq root	log
		P value	P value	P value	P value
Inner Lagoon	21	0.015*	0.211	0.026*	0.254
Outer Lagoon	21	0.270	0.204	0.236	0.132
Rim Reef	21	0.005*	0.062	0.006*	0.024*

2. 2. The number of planula released cm^{-2} on each day from colonies collected at the Inner Lagoon, Outer Lagoon and Rim Reef (data for each zone is the mean number of planulae released from all colonies monitored from July and August 1999 and 2000). N= 21 days of monitoring for planula release (10 days either side of the new moon).

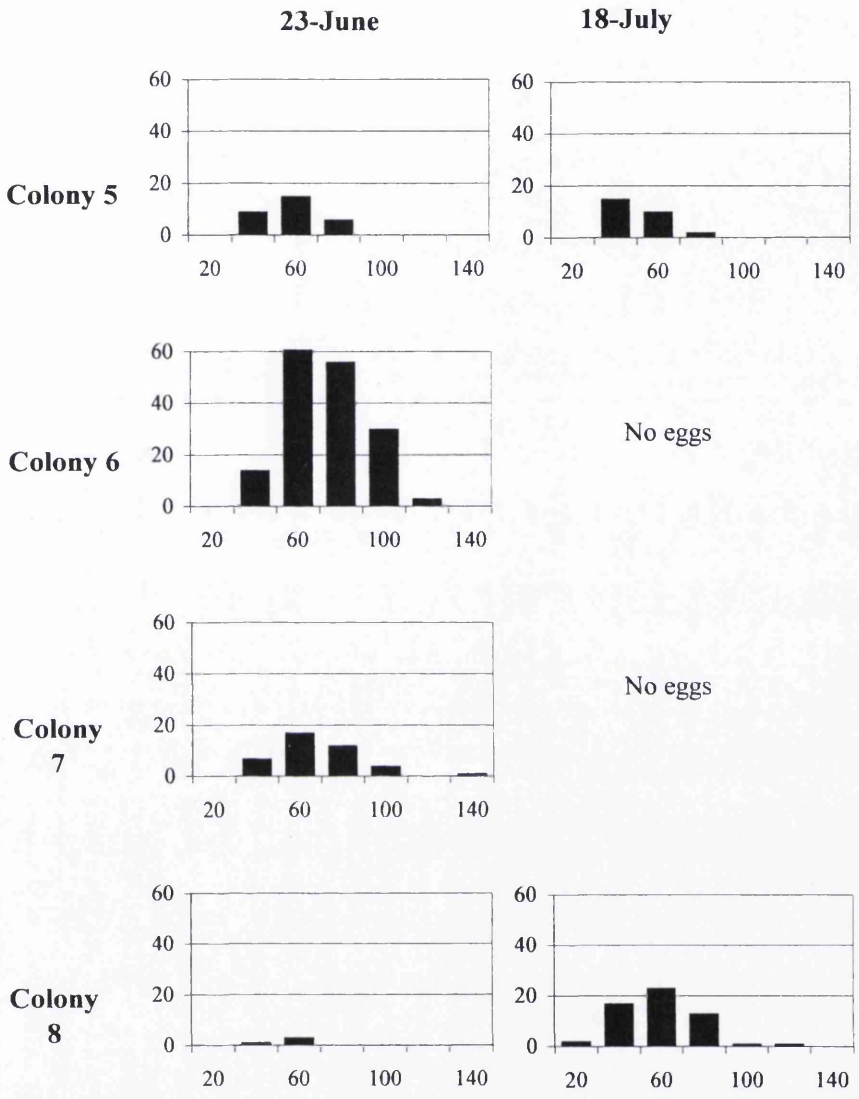
* is a non-significant result ($P>0.05$), indicating that the data are normally distributed.

Sample	n	raw data	arc sine	sq root	log
		P value	P value	P value	P value
Inner Lagoon	21	0.041*	0.707	0.058	0.326
Outer Lagoon	21	0.003*	0.497	0.004*	0.049*
Rim Reef	21	0.000*	0.103	0.001*	0.009*

Appendix 3.5A: ***Porites astreoides.*** **Size frequency distributions of egg diameter from Crescent Reef colonies 1-4**



Appendix 3.5B: ***Porites astreoides.*** **Size frequency distributions of egg diameter from Crescent Reef colonies 5-8**



Appendix 4.1A: Sampling dates of tagged *Pseudoplexaura porosa* colonies at the Outer Lagoon and Rim Reef to monitor gamete development in 1998 (section 4.3.3). Five colonies were sampled from each site (except on April 15).

The sample dates just before spawning, around the full moon, of May-October were used to determine the months of spawning (section 4.3.4) and fecundity and polyp volume (section 4.3.5). The samples used to determine *P. porosa* gamete maturation and reproductive effort for Chapter 5 are those just before spawning in July-October.

Crescent C, Outer Lagoon	Hog Breaker, Rim Reef	
Date	Date	
April 15 *	April 15 *	* 8 random colonies
May 7	May 7	
May 16	May 16	
May 29	May 29	
June 11	June 11	
June 24	June 24	
July 6	July 7	
July 14	July 15	
Aug 5	Aug 5	
Aug 21		
Sep 4	Sep 4	
Sep 11	Sep 11	
Sep 16	Sep 14	
Sep 30	Sep 30	
Oct 20	Oct 20	
Nov 11	Nov 17	

Full moon dates of potential spawning months in 1998:

July 9

August 7

September 6

October 5

November 5

Appendix 4.1B: Sample dates of tagged *Pseudoplexaura porosa* colonies at the three reef zones to monitor the months of spawning in 1999 and 2000 (section 4.3.4; samples for 1998 were those collected around the full moon in Appendix 4.1A). The table shows the date that each site was visited.

The samples used to determine *P. porosa* gamete maturation and reproductive effort for Chapter 5 are July-October each year.

1999

Month	Date of full moon	Inner Lagoon		Outer Lagoon		Rim Reef	
		TW	TE	C	B	H	T
May	30	27	27	2June	-	25	25
June	28	28	28	22	-	30	30
July	28	26	26	28	-	29	29
August	26	27	27	26	26	25	20
September	25	27	27	26	26	29	29
October	24	14	14	20	26	15	15

2000

Month	Date of full moon	Inner Lagoon		Outer Lagoon		Rim Reef	
		TW	TE	C	B	H	T
May	18	22	22	23	23	16	16
June	16	13	13	15	15	14	14
July	16	19	19	18	18	20	20
August	15	16	16	19	19	18	17
September	13	14	14	12	12	13	13
October	13	10	10	13	13	11	11

Appendix 4.1C: **The number of *Pseudoplexaura porosa* colonies of each sex sampled from the study sites in 1999 and 2000.**

1999	Inner Lagoon		Outer Lagoon		Rim Reef	
	TW	TE	C	B	H	T
Male	3	3	3	2	3	4
Female	5	4	4*	4	5	3*
Total	8	7	7	6	8	7

2000	Inner Lagoon		Outer Lagoon		Rim Reef	
	TW	TE	C	B	H	T
Male	2	3	2	2	2	3
Female	4	3	3	4	5*	3
Total	6	6	5	6	7	6

*one female colony not sampled

Appendix 4.2: **The occurrence of *Pseudoplexaura porosa* spicules in the tagged colonies. Replicate spicule prepartions (A and B) were made from each tagged colony.**

Abundance scale:		
	1	Rare
	2	Occasional
	3	Common
	4	Frequent
	5	Abundant

TYNES WEST

Colony Tag	W1		W2		W3		W4		W5		W6		W7		W8	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Spindle	4	4	5	4	4	5	5	5	5	5	5	5	5	5	5	5
Spiney spindle	2	2				2	1	2	1		2	1	1		2	2
Clubs	3	2	3	4	2	2	1			1	1	1	1	1	1	1
purple spindle	1	1	5	4	2	1	3	4	4	5	4	4	4	4	3	3
rayed purple		1				1	1	1	2	2	1	1		1		2

TYNES EAST

Colony Tag	E1		E2		E4		E5		E6		E7		E8	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Spindle	5	5	4	5	5	4	3	3	3	3	4	4	4	4
Spiney spindle	1	1		1		1	1		1		1		1	1
Clubs		1		1	1	2	2	2	2	1	2	2	1	2
purple spindle	2	4	3	3	3	2	5	4	4	3	3	2	4	4
rayed purple	2	1	1	1			1	1	1	1		1		

CRESCENT C

Colony Tag	C1		C2		C3		C4		C5		C6		C8	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Spindle	5	5	5	5	4	4	5	5	4	5	4	4	4	4
Spiney spindle	1		1		1			1	1			1	2	1
Clubs	1	2	1	2	2	2	3	2	2	2	3	1	3	1
purple spindle	4	4	4	4	5	5	3	3	3	3	3	4	4	2
rayed purple			1	2		1		1	1	2				

CRESCENT B

Colony Tag	B1		B2		B3		B4		B6		B7	
	A	B	A	B	A	B	A	B	A	B	A	B
Spindle	5	5	5	5	5	5	4	4	4	5	5	5
Spiney spindle						2		1		1	1	
Clubs	3	4	3	4	1	2	1	2	2	2	1	2
purple spindle	3	4	4	2	2	4	2	2	2	2	2	3
rayed purple	3	1	1	1	1	2						1

HOG BREAKER

Colony Tag	H1		H2		H3		H4		H5		H6		H7		H8	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Spindle	5	5	5	5	4	5	5	5	5	5	5	5	4	5	5	5
Spiney spindle		1	1	2	1		1	1	2					1	1	
Clubs	3	3	2	3	2	2	3	3	3	5	3	3	2	2	3	4
purple spindle	2	4	2	4	4	3	3	2	3	4	3	3	4	2	4	4
rayed purple		1				1	1	1			1				3	2

TWIN BREAKER

Colony Tag	T1		T2		T3		T4		T5		T7		T8	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Spindle	5	5	5	5	5	4	4	4	5	3	4	4	5	5
Spiney spindle	1		1	1	1		1			1			1	
Clubs	3	2	2	1	2	2	2	2	3	2	1	4	3	2
purple spindle	4	3	3	3	3	4	4	2	3	4	2	1	2	3
rayed purple	1	1		1	2		1	1					3	1

SUMMARY	count	% occurrence	# colonies	% occurrence
Spindle	86	100	43	100
Spiney spindle	48	56	38	88
Clubs	82	95	43	100
purple spindle	86	100	43	100
rayed purple	47	55	30	70

Total preparations: 86 Total number of colonies 43

Appendix 4.3: Mean polyp volume of *Pseudoplexaura porosa* colonies: testing for normality of the data and homogeneity of variances prior to ANOVA.

1. Kolmogorov-Smirnov test for normality (Sokal and Rohlf, 1995) performed by the statistical package SYSTAT 5.2.

Variable	n	P
Tynes West	16	1.000
Tynes East	14	0.425
Crescent C	16	0.051
Crescent B	14	1.000
Hog Breaker	14	0.622
Twin Breaker	12	0.081

All samples are normally distributed ($P>0.05$)

2. Bartlett's test for homogeneity of variances (Sokal and Rohlf 1995) performed using the statistical package BIOMstat 3.

$\chi^2 = 1.4109$, $df = 5$

$c = 1.0295$

$\chi^2 c = 1.3705$, $P = 0.9275$

Variances are homogeneous ($P>0.05$)

Appendix 4.4: Mean polyp volume of *Pseudoplexaura porosa* colonies: multiple comparison of means testing after ANOVA.

Samples are the mean polyp volume from replicate sites of the three reef zones:

1 Tynes West, Inner Lagoon

2 Tynes East, Inner Lagoon

3 Crescent C, Outer Lagoon

4 Crescent B, Outer Lagoon

5 Hog Breaker, Rim Reef

6 Twin Breaker, Rim Reef

The Tukey-Kramer (TK) and GT2 and T tests were performed.

* indicates P<=0.050 for at least one method.

Comparing sample 5 against:

Sample:	3	6	4	2	1
Diff:	3.2817*	4.5846*	5.9816*	7.6060*	8.4674*
MSD(TK):	3.1662	3.1662	3.3040	3.1662	3.0589
MSD(GT2):	3.2665	3.2665	3.4086	3.2665	3.1558
MSD(T'):	3.2701	3.2701	3.5321	3.2701	3.0589

Comparing sample 3 against:

Sample:	6	4	2	1
Diff:	1.3029	2.6998	4.3242*	5.1857*
MSD(TK):	3.2701	3.4036	3.2701	3.1662
MSD(GT2):	3.3737	3.5114	3.3737	3.2665
MSD(T'):	3.2701	3.5321	3.2701	3.2701

Comparing sample 6 against:

Sample:	4	2	1
Diff:	1.3969	3.0214	3.8828*
MSD(TK):	3.4036	3.2701	3.1662
MSD(GT2):	3.5114	3.3737	3.2665
MSD(T'):	3.5321	3.2701	3.2701

Comparing sample 4 against:

Sample:	2	1
Diff:	1.6244	2.4859
MSD(TK):	3.4036	3.3040
MSD(GT2):	3.5114	3.4086
MSD(T'):	3.5321	3.5321

Comparing sample 2 against:

Sample:	1
Diff:	0.8614
MSD(TK):	3.1662
MSD(GT2):	3.2665
MSD(T'):	3.2701

Appendix 4.5: *Pseudoplexaura porosa* mean polyp volume versus gamete volume: testing for normality of the mean oocyte and spermary volumes prior to correlation statistics.

Kolmogorov-Smirnov test for normality (Sokal and Rohlf, 1995) performed by the statistical package SYSTAT 5.2.
Transformations tested were arc sine (sin-1($\sqrt{Y}/100$)), log ($\log_{10}$) and square root ($\sqrt{Y}$)

*is a significant result (P>0.05) indicating that the data are not normally distributed

Variable	n	raw data	arc sine	log	square root
Oocyte volume	24	0.000*	0.052	0.003*	0.000*
Spermary volume	18	0.405	1.000	0.750	0.672

Arc sine transformation of the oocyte volume data was necessary for a normal distribution (P>0.05). The spermary volume data are similarly transformed prior to the correlation statistics.

Appendix 5.1: ***Porites astreoides.*** **Testing for homogeneity of variances among numbers of planulae released per cm^2 per day from colonies collected at the three reef zones in Bermuda over the summer months of 1999 and 2000.**

Bartlett's test for homogeneity of variances (Sokal and Rohlf, 1995) performed by the statistical package BIOMstat 3.

Total sample size = 146

Total sample size of means= 30 (see Appendix 5.5 for raw data)

χ^2 = 110.2661, df = 29

c = 1.089939

χ^2c = 101.1672, P = 6.358 10^-10

The variances of the 30 samples are heterogeneous (P<0.05)

The following transformations on the data were tested for their effectiveness at removing variance heterogeneity:

Square root transformation ($\sqrt{(Y+0.5)}$)

Bartlett's test:

χ^2 = 677.6327, df = 29

c = 1.089939

χ^2c = 621.7160, P = 0

The variances of the 30 samples are heterogeneous (P<0.05)

Log to base 10 ($\log_{10}$)

Bartlett's test:

χ^2 = 64.1024, df = 29

c = 1.089939

χ^2 c = 58.8129, P = 0.0009

The variances of the 30 samples are heterogeneous (P<0.05)

Arc sine of square root (sin-1(√Y/100))

Bartlett's test:

χ^2 = 34.2510, df = 29

c = 1.089939

χ^2 c = 31.4246, P = 0.3457

The variances of the 30 samples are homogeneous (P>0.05)

Appendix 5.2A: *Pseudoplexaura porosa* oocytes.

Testing for homogeneity of variances among measures of oocyte volume per polyp from colonies collected at replicate sites from the three reef zones in Bermuda over the summer months of 1999 and 2000.

Bartlett's test for homogeneity of variances (Sokal and Rohlf, 1995) performed using the statistical package BIOMstat 3.

Total sample size = 87

Total sample size of means= 23 (see Appendix 5.6A for raw data)

Bartlett's test:

χ^2 = 47.5691, df = 22

c = 1.139915

χ^2 c = 41.7304, P = 0.0067

The variances of the 23 samples are heterogeneous ($P<0.05$)

The following transformations on the data were tested for their effectiveness at removing variance heterogeneity:

Square root transformation ($\sqrt{(Y+0.5)}$)

Bartlett's test:

χ^2 = 45.4395, df = 22

c = 1.139915

χ^2 c = 39.8622, P = 0.0112

The variances of the 23 samples are heterogeneous ($P<0.05$)

Log to base 10 ($\log_{10}$)

Bartlett's test:

χ^2 = 45.3433, df = 22

c = 1.139915

χ^2 c = 39.7778, P = 0.0115

The variances of the 23 samples are heterogeneous ($P<0.05$)

Arc sine of square root of Y/100 (sin-1(√Y/100))

Bartlett's test:

χ^2 = 19.7485, df = 22

c = 1.139915

χ^2 c = 17.3256, P = 0.7452

The variances of the 23 samples are homogeneous (P>0.05)

B: *Pseudoplexaura porosa* spermaries

Testing for homogeneity of variances among measures of spermary volume per polyp from colonies collected at replicate sites from the three reef zones in Bermuda over the summer months of 1999 and 2000.

Bartlett's test for homogeneity of variances (Sokal and Rohlf, 1995) performed by the statistical package BIOMstat 3.

Total sample size = 64

Total sample size of means= 24 (see Appendix 5.6B for raw data)

Bartlett's test:

χ^2 = 50.5527, df = 23

c = 1.241184

χ^2 c = 40.7295, P = 0.0127

The variances of the 24 samples are heterogeneous (P<0.05)

The following transformations on the data were tested for their effectiveness at removing variance heterogeneity:

Square root transformation ($\sqrt{(Y+0.5)}$)

Bartlett's test:

χ^2 = 50.1494, df = 23

c = 1.241184

χ^2 c = 40.4045, P = 0.0138

The variances of the 24 samples are heterogeneous (P<0.05)

Log to base 10 ($\log_{10}$)

Bartlett's test:

χ^2 = 50.1426, df = 23

c = 1.241184

χ^2 c = 40.3991, P = 0.0139

The variances of the 24 samples are heterogeneous (P<0.05)

Arc sine of square root (sin-1(√Y/100))

Bartlett's test:

χ^2 = 42.3439, df = 23

c = 1.241184

χ^2 c = 34.1157, P = 0.0635

The variances of the 24 samples are homogeneous (P>0.05)

Appendix 5.3: ***Porites astreoides.*** **Testing for normality of the data**

Kolmogorov-Smirnov test (Sokal and Rohlf, 1995) performed by the statistical package SYSTAT 5.2.

See Appendix 5.5 for raw data

No planulae were released from the Inner Lagoon in September 1999 and 2000 and from the Outer Lagoon in September 1999, and so the samples are not included.

*is a significant result (P>0.05) indicating that the data are not normally distributed

		Non-transformed		Arc sine transformation	
Sample	n	maxdif	P value	maxdif	P value
Inner Lagoon Jul99	5	0.296	0.190	0.256	0.459
Outer Lagoon Jul99	5	0.326	0.090	0.334	0.070
Rim Reef Jul99	5	0.301	0.167	0.257	0.448
Inner Laggon Aug99	10	0.405	0.000*	0.258	0.057
Outer Lagoon Aug99	8	0.220	0.347	0.152	1.000
Rim Reef Aug99	8	0.313	0.020*	0.213	0.406
Rim Reef Sep99	5	0.408	0.007*	0.328	0.084
Inner Lagoon Jul00	10	0.348	0.001*	0.213	0.240
Outer Lagoon Jul00	10	0.377	0.000*	0.277	0.028*
Rim Reef Jul00	10	0.232	0.139	0.162	0.768
Inner Lagoon Aug00	10	0.329	0.003*	0.256	0.063
Outer Lagoon Aug00	10	0.211	0.251	0.123	1.000
Rim Reef Aug00	10	0.238	0.115	0.188	0.450
Outer Lagoon Sep00	10	0.480	0.000*	0.482	0.000*
Rim Reef Sep00	10	0.256	0.459	0.249	0.079

The data from the Outer Lagoon July 2000 and the Outer Lagoon September 2000 samples were not successfully transformed to normality.

Appendix 5.4A: *Pseudoplexaura porosa* oocytes. Testing for normality of the data

Kolmogorov-Smirnov test (Sokal and Rohlf, 1995) performed by the statistical package SYSTAT 5.2.

See Appendix 5.6 for raw data

*is a significant result (P>0.05) indicating that the data are not normally distributed.

		Non-transformed		Arc sine transformation	
Sample	n	maxdif	P value	maxdif	P value
Inner Lagoon Jul99	8	0.184	0.728	0.149	1.000
Outer Lagoon Jul99	3	0.226	1.000	0.264	1.000
Rim Reef Jul99	7	0.161	1.000	0.242	0.282
Inner Lagoon Aug99	8	0.179	0.798	0.125	1.000
Outer Lagoon Aug99	8	0.247	0.172	0.129	1.000
Rim Reef Aug99	8	0.240	0.210	0.161	1.000
Inner Lagoon Jul00	7	0.148	1.000	0.191	0.802
Outer Lagoon Jul00	7	0.338	0.015*	0.265	0.159
Rim Reef Jul00	7	0.202	0.659	0.168	1.000
Inner Lagoon Aug00	7	0.332	0.019	0.386	0.052
Outer Lagoon Aug00	7	0.289	0.079	0.195	0.745
Rim Reef Aug00	8	0.245	0.182	0.225	0.303

All Inner Lagoon August 2000 data were successfully transformed to normality.

B: *Pseudoplexaura porosa* spermaries

Testing for normality of the data

Kolmogorov-Smirnov test (Sokal and Rohlf, 1995) performed by the statistical package SYSTAT 5.2.

See Appendix 5.6 for raw data

		Non-transformed		Arc sine transformation	
Sample	n	maxdif	P value	maxdif	P value
Inner Lagoon Jul99	6	0.240	0.421	0.245	0.378
Outer Lagoon Jul99	5	0.278	0.289	0.261	0.414
Rim Reef Jul99	6	0.247	0.363	0.259	0.278
Inner Lagoon Aug99	6	0.172	1.000	0.199	0.893
Outer Lagoon Aug99	5	0.372	0.022	0.309	0.140
Rim Reef Aug99	6	0.172	1.000	0.165	1.000
Inner Lagoon Jul00	5	0.353	0.041*	0.318	0.111
Outer Lagoon Jul00	4	0.263	0.636	0.246	0.837
Rim Reef Jul00	5	0.266	0.371	0.197	1.000
Inner Lagoon Aug00	5	0.181	1.000	0.223	0.814
Outer Lagoon Aug00	4	0.223	1.000	0.237	0.958
Rim Reef Aug00	5	0.228	0.753	0.304	0.158

All Inner Lagoon July 2000 data were successfully transformed to normality.

Appendix 5.5: Number of *Porites astreoides* planulae released per cm^2 per day from colonies collected at the Inner Lagoon, Outer Lagoon and Rim Reef zones over July to September 1999 and 2000 (replicate study sites were sampled in August 1999 and all months in 2000).

Zone	Reef	Colony	Jul-99	Aug-99	Sep-99	Jul-00	Aug-00	Sep-00
Inner	TW	TW1	0.0100	0.0000	0.0000	0.0405	0.0059	0.0000
Lagoon		TW2	0.0656	0.0094	0.0000	0.1459	0.0021	0.0000
		TW3	0.0069	0.0000	0.0000	0.0000	0.0225	0.0000
		TW4	0.0196	0.0013	0.0000	0.0182	0.0065	0.0000
		TW5	0.1191	0.0006	0.0000	0.0169	0.0628	0.0000
Inner	TE	TE1		0.0152		0.0000	0.0057	0.0000
Lagoon		TE2		0.0000		0.0122	0.0056	0.0000
		TE3		0.0000		0.0008	0.0156	0.0000
		TE4		0.0008		0.0003	0.0009	0.0000
		TE5		0.0000		0.0014	0.0000	0.0000
Outer	C	C1	0.0566	0.0086	0.0000	0.0009	0.0242	0.0000
Lagoon		C2	0.0000	0.0048	0.0000	0.0170	0.1674	0.0000
		C3	0.1825	0.0000	0.0000	0.0454	0.0575	0.0000
		C4	0.0008	0.0173	0.0000	0.0288	0.0527	0.0035
		C5	0.0007		0.0000	0.0252	0.0626	0.0000
Outer	B	B1		0.0055		0.0024	0.0000	0.0000
Lagoon		B2		0.0031		0.0244	0.0059	0.0000
		B3		0.0016		0.0070	0.0101	0.0000
		B4		0.0000		0.2436	0.1018	0.0000
		B5				0.0189	0.0284	0.0026
Rim	H	H1	0.0685	0.0036	0.0000	0.1086	0.1646	0.0000
Reef		H2	0.0057	0.0452	0.0033	0.0802	0.0093	0.0000
		H3	0.0344	0.0014	0.0000	0.0017	0.1859	0.0000
		H4	0.0016	0.0000	0.0336	0.0210	0.0259	0.0000
		H5	0.0090		0.0000	0.0000	0.1812	0.0250
Rim	T	T1		0.0000		0.0277	0.3997	0.0008
Reef		T2		0.0116		0.0000	0.2938	0.0009
		T3		0.0000		0.0475	0.0031	0.0023
		T4		0.0547		0.0056	0.0057	0.0034
		T5				0.0000	0.0436	0.0309

Appendix 5.6A: Female colonies

Total volume of *Pseudoplexaura porosa* oocytes (>500μm) per polyp within colonies sampled from replicate reefs (except in July 1999 from the Outer Lagoon) at the Inner Lagoon, Outer Lagoon and Rim Reef zones over July and August 1999 and 2000.

Also shown is the mean polyp volume of each colony that is later used to normalise the reproductive effort per polyp (see text for details).

Zone	Reef	Coral	polyp volume	Jul-99	Aug-99	Jul-00	Aug-00
Inner	TW	TW1	12.81	0.9377	1.2039	0.6626	0.6013
Lagoon		TW2	16.06	0.4682	0.8784	0.6421	0.5779
		TW3	12.41	0.0436	0.2540		
		TW4	11.85	0.0606	0.7133	0.0000	0.0000
		TW5	12.55	0.5089	0.1576	0.0000	0.0000
Inner	TE	TE1	10.70	0.1560	1.6057	0.2548	0.3976
Lagoon		TE2	10.14	0.2422	0.4664	1.1792	0.3811
		TE3	14.21	1.0949	1.3068	1.0345	0.7124
		TE4	9.34	0.1817	0.1602		
Outer	C	C1	9.50	0.0723	0.2279	0.1145	0.0508
Lagoon		C2	8.35	0.0144	0.0000	0.0087	0.3513
		C3	7.60	0.0405	0.6574	0.0087	0.0431
		C4	8.49		0.4580		
Outer	B	B1	12.44		0.0586	0.0796	0.1911
Lagoon		B2	13.14		1.5811	0.7376	1.0119
		B3	5.57		0.0000	0.0000	0.0000
		B4	10.76		0.1566	0.0087	0.0000
Rim	H	H1	7.59	0.2052	0.0144	0.0000	0.0000
Reef		H2	6.02	0.0610	0.6358	0.0000	0.0518
		H3	8.28	0.1934	0.0857	0.0452	0.1094
		H4	7.89	0.0000	0.1880		0.0000
		H5	7.09	0.3267	0.3107	0.1094	0.0683
Rim	T	T1	5.80	0.0000	0.1232	0.0087	0.2209
Reef		T2	7.96	0.2378	0.3494	0.0806	0.2763
		T3	8.65		0.0693	0.1797	0.0000

B: Male colonies

Total volume of *Pseudoplexaura porosa* spermaries per polyp within colonies sampled from replicate reefs at the Inner Lagoon, Outer Lagoon and Rim Reef zones over July and August 1999 and 2000.

Also shown is the mean polyp volume of each colony that is later used to normalise the reproductive effort per polyp (see text for details).

Zone	Reef	Coral	polyp volume	Jul-99	Aug-99	Jul-00	Aug-00
Inner	TW	TW1	8.99	0.1058	0.1967		
Lagoon		TW2	15.54	0.1603	0.1272	0.2977	0.1122
		TW3	13.52	0.1396	0.1957	0.0149	0.1372
Inner	TE	TE1	14.62	0.1080	0.2298	0.3627	0.0304
Lagoon		TE2	10.71	0.0549	0.0764	0.0005	0.1586
		TE3	14.71	0.1079	0.1978	0.0175	0.0976
Outer	C	C1	5.79	0.0295	0.0164	0.0065	0.1137
Lagoon		C2	10.19	0.0777	0.0778	0.0039	0.1836
		C3	4.24	0.0309	0.0530		
Outer	B	B1	12.16	0.1452	0.1534	0.0601	0.1709
Lagoon		B2	8.39	0.1531	0.3454	0.0545	0.0597
Rim	H	H1	6.86	0.0273	0.0166	0.0006	0.0042
Reef		H2	6.13	0.0862	0.0935	0.0361	0.0657
		H3	7.01	0.0980	0.0304		
Rim	T	T1	8.84	0.0471	0.1194	0.0105	0.0517
Reef		T2	11.90	0.0744	0.1248	0.0152	0.0659
		T3	6.06	0.0753	0.1518		
		T4	14.03	0.0690	0.1848	0.0389	0.1177

Appendix 5.7: ***Porites astreoides*: multiple comparison among means**

Samples are the number of planulae per cm^2 per day collected from colonies collected at the three reef zones in Bermuda over the summer months of 1999 and 2000 (see Appendix 5.5 for raw data). The arc sine transformation was performed prior to the statistical testing (Appendix 5.1 and 5.3).

Sample number and corresponding month and reef zone:

Month		**Reef zone**	
1	July 99	1	Inner Lagoon
2	August 99	2	Outer Lagoon
3	September 99	3	Rim Reef
4	July 00		
5	August 00		
6	September 00		

The Tukey-Kramer (TK), GT2 and T′ tests were performed.

* indicates P<=0.050 for at least one method.

Month

Comparing sample 3 against:

Sample:	6	2	4	1	5
Diff:	0.0243	0.2219	0.6571*	0.7873*	1.0337*
MSD(TK):	0.5682	0.5826	0.5682	0.6561	0.5682
MSD(GT2):	0.5852	0.6000	0.5852	0.6757	0.5852
MSD(T'):	0.6561	0.6561	0.6561	0.6561	0.6561

Comparing sample 6 against:

Sample:	2	4	1	5
Diff:	0.1976	0.6328*	0.7631*	1.0094*
MSD(TK):	0.4814	0.4639	0.5682	0.4639
MSD(GT2):	0.4958	0.4778	0.5852	0.4778
MSD(T'):	0.4983	0.4639	0.6561	0.4639

Comparing sample 2 against:

Sample:	4	1	5
Diff:	0.4352	0.5655	0.8118*
MSD(TK):	0.4814	0.5826	0.4814
MSD(GT2):	0.4958	0.6000	0.4958
MSD(T'):	0.4983	0.6561	0.4983

Comparing sample 4 against:

Sample:	1	5
Diff:	0.1303	0.3766
MSD(TK):	0.5682	0.4639
MSD(GT2):	0.5852	0.4778
MSD(T'):	0.6561	0.4639

Comparing sample 1 against:

Sample:	5
Diff:	0.2463
MSD(TK):	0.5682
MSD(GT2):	0.5852
MSD(T'):	0.6561

Zone

Comparing sample 1 against:

Sample:	2	3
Diff:	0.2158	0.3921*
MSD(TK):	0.3426	0.3426
MSD(GT2):	0.3494	0.3494
MSD(T'):	0.3468	0.3468

Comparing sample 2 against:

Sample:	3
Diff:	0.1763
MSD(TK):	0.3461
MSD(GT2):	0.3529
MSD(T'):	0.3468

Appendix 5.8: *Pseudoplexaura porosa*: multiple comparison among means

Samples are the volume of oocyte of spermaries per polyp (normalised to mean polyp volume) from colonies at the three reef zones in Bermuda just prior to spawning on the summer months of 1999 and 2000 (see Appendix 5.6 for raw data). The arc sine transformation was performed prior to the statistical testing (Appendix 5.2 and 5.4).

Sample number and corresponding month and reef zone:

Month		**Reef zone**	
1	July 99	1	Inner Lagoon
2	August 99	2	Outer Lagoon
3	July 00	3	Rim Reef
4	August 00		

The Tukey-Kramer (TK), GT2 and T′ tests were performed.

* indicates P<=0.050 for at least one method.

Oocytes: comparison of the monthly means

Comparing sample 3 against:

Sample:	4	1	2
Diff:	0.0415	0.1467	0.4491*
MSD(TK):	0.4437	0.4605	0.4305
MSD(GT2):	0.4556	0.4728	0.4420
MSD(T'):	0.4490	0.4720	0.4490

Comparing sample 4 against:

Sample:	1	2
Diff:	0.1053	0.4077
MSD(TK):	0.4555	0.4252
MSD(GT2):	0.4677	0.4365
MSD(T'):	0.4720	0.4386

Comparing sample 1 against:

Sample:	2
Diff:	0.3024
MSD(TK):	0.4426
MSD(GT2):	0.4545
MSD(T'):	0.4720

Oocytes: comparison of the means of each zone

Comparing sample 2 against:

Sample:	3	1
Diff:	0.0035	0.4123*
MSD(TK):	0.3529	0.3479
MSD(GT2):	0.3601	0.3549
MSD(T'):	0.3693	0.3693

Comparing sample 3 against:

Sample:	1
Diff:	0.4087*
MSD(TK):	0.3312
MSD(GT2):	0.3379
MSD(T'):	0.3371

Spermaries: comparison of the monthly means

Comparing sample 3 against:

Sample:	4	1	2
Diff:	0.2041*	0.2130*	0.3103*
MSD(TK):	0.1965	0.1852	0.1852
MSD(GT2):	0.2019	0.1904	0.1904
MSD(T'):	0.1965	0.1965	0.1965

Comparing sample 4 against:

Sample:	1	2
Diff:	0.0089	0.1061
MSD(TK):	0.1852	0.1852
MSD(GT2):	0.1904	0.1904
MSD(T'):	0.1965	0.1965

Comparing sample 1 against:

Sample:	2
Diff:	0.0972
MSD(TK):	0.1733
MSD(GT2):	0.1781
MSD(T'):	0.1733

Spermaries: comparison of the means of each zone

Comparing sample 3 against:

Sample:	1	2
Diff:	0.0684	0.0891
MSD(TK):	0.1574	0.1663
MSD(GT2):	0.1607	0.1697
MSD(T'):	0.1611	0.1781

Comparing sample 1 against:

Sample:	2
Diff:	0.0208
MSD(TK):	0.1695
MSD(GT2):	0.1730
MSD(T'):	0.1781

Appendix 6.1: Survival of *Madracis mirabilis* colonies across the reef zones of the Bermuda platform: an exploratory transplant experiment

The natural occurrence of *Madracis mirabilis* colonies in Bermuda is limited to the shallow lagoonal patch reefs of the Inner Lagoon and Outer Lagoon and the offshore deep Terrace Reef, and is rare along the intermediate Rim Reef zone (Chapter 6). Branches of *M. mirabilis* were transplanted to replicate sites at the Rim Reef zone to monitor their survival (Figure 2.1, Chapter 2 for location of the study sites). Five racks of approximately 20 sq. cm were constructed of plastic gridding and secured to the reef using nails. Five racks were also deployed at the replicate sites at the Outer Lagoon reef zone (*M. mirabilis* common) and at the Inner Lagoon reef zone (*M. mirabilis* abundant). The Terrace reef was not included in this preliminary study. Over the summer of 1998 between 5 and 10 healthy *M. mirabilis* branches with 100 % coral coverage were collected from the Inner Lagoon reef zone and attached to each rack at all the sites by cable ties. The racks were monitored approximately one year later (October and November 1999) and each of the branches were recorded for percent live coral, percent skeleton, percent algae coverage, or were scored as total mortality.

The racks at the Inner Lagoon sites had the largest percentage of branches with >50% coral tissue cover with only a small number of branches that were mainly skeleton or dominated by algae (Figure 6.1.1). The health of the transplanted *M. mirabilis* branches at the Outer Lagoon reef zone was variable between the two replicate sites. At Crescent B, over 70% of the branches had >50% coral coverage, a similar survival to the transplanted branches at the Inner Lagoon reef zone. In contrast, the greatest percentages of branches on the racks at Crescent C were dominated by skeleton. The transplanted *M. mirabilis* branches at the replicate sites of the Rim Reef zone showed intermediate levels of coral tissue survival. The percentage of branches dominated by coral tissue was 9% greater at Twin Breaker than at Hog Breaker. The percentage of branches dominated by algal coverage did not vary at the different reef zones. Total mortality occurred for 8-12% of the branches at the Outer Lagoon sites and 4% of the branches at Twin Breaker of the Rim Reef zone. All transplanted branches survived at the Inner Lagoon sites. There were no observational differences in growth rate of the transplanted branches between the different sites. However, accurate growth

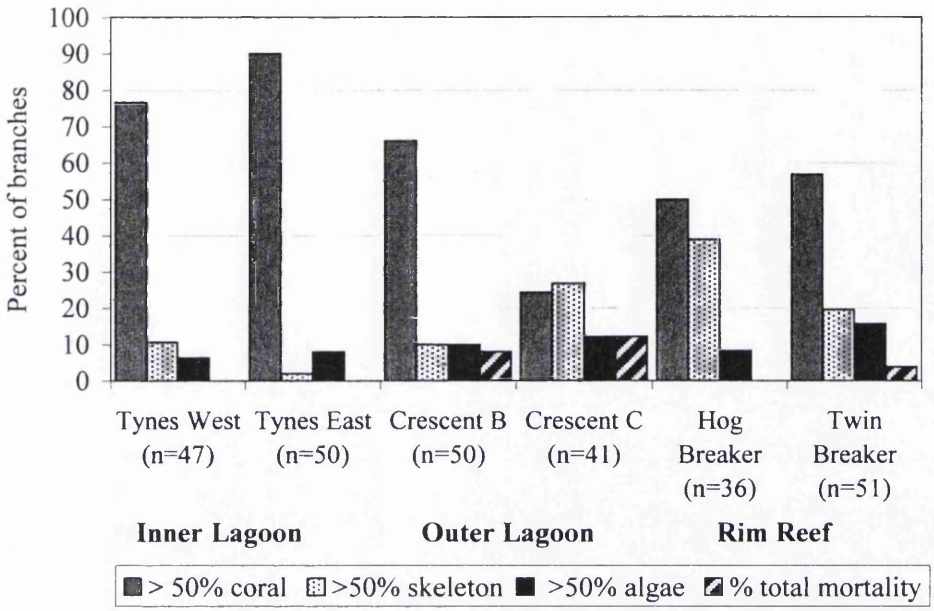


Figure 6.1.1: The percentage of *Madracis mirabilis* branches on transplanted racks with >50% coverage of coral, skeleton and algae, and the percentage of the branches that died, at the replicate study sites from the three zones of the North Lagoon. The original number of branches transplanted (n) is shown on the x axis.

measurements are needed, such as the use of a staining technique (Lamberts, 1978) or buoyant weight determination (Spencer Davies, 1989).

The survival of the transplanted *Madracis mirabilis* fragments to the different reef zones of the Bermuda platform followed the natural distribution of the species. Survival is greatest at the Inner Lagoon where *M. mirabilis* is naturally abundant, variable at the Outer Lagoon where natural occurrence is patchy and the lowest survival occurred at the outer Rim Reef where colonies are naturally rare. The growth and survival of transplanted coral fragments has been shown to be influenced by substrate composition (Heyward and Collins, 1985), macroalgal cover (Yap *et al.*, 1998), wave energy (Plucer-Rosario and Randall, 1987), sedimentation (Yap and Gomez, 1985; Nagelkerken *et al.*, 2000), light intensity (Yap *et al.*, 1998), and seawater temperature (Yap and Gomez, 1985). The substrate composition is relatively similar at each reef zone with the transplanted racks always placed onto rock on the reef above the sand. Macroalgal abundance in Bermuda is patchy and highly seasonal and inter- or intra-zone patterns in abundance do not occur (S.R. Smith, pers. comm.). The occurrence of macroalgae on the transplanted fragments was minimal (<20 %) at all the sites and is not believed to be an important factor in the variable survival of the coral branches. The important factors believed to be limiting *M. mirabilis* survival are the gradients of sedimentation and light levels, and variability in wave energy across the Bermuda reef zones (see Chapter 2 for a description of the environmental variation across the reef zones).

Sedimentation and associated turbidly levels are greatest inshore at the Inner Lagoon, moderate at the Outer Lagoon and lowest at the Rim Reef (Bodungen *et al.*, 1982; CARICOMP, 1997; Chapter 2). Highest sedimentation rates are normally related to greatest mortality of transplanted corals (Nagelkerken *et al.*, 2000). However, in Bermuda the branching *M. mirabilis* colonies that flourish inshore are clearly adapted to the high sedimentation levels. The lower sedimentation levels offshore should be favourable to the colonies and not detrimental, and colonies do occur along the Terrace reef were sedimentation is low, and so the effect of sedimentation directly is not believed to be affecting fragment survival. The levels of sediment in the water will secondarily effect light levels, which are therefore low inshore and light attenuation is greater offshore at reefs of a similar depth, such as the Rim Reef. The shade-adapted

M. mirabilis fragments from the Inner Lagoon may be light stressed after transplantation to the clearer, shallow offshore waters of the Rim Reef zone. *M. mirabilis* colonies are found at the deeper Terrace reef (>20m), where light attenuation will be reduced compared to the shallower Rim Reef. Colonies growing at the Terrace Reef additionally avoid high light by being fairly cryptic and are often confined to shaded overhangs and cave entrances (T. Murdoch, pers. comm.). The sheltered Inner Lagoon and the deep Terrace Reef are also similar in being low energy environments (except during periodic storms), compared to the high wave and surge impacted shallow offshore Rim Reef.

The populations of *M. mirabilis* along the inshore shallow lagoonal reefs and the offshore deeper Terrace reefs do differ in respect to environmental variation in nutrient availability. Nutrient levels are greater in the inshore environment compared to offshore, which may limit *M. mirabilis* distribution (Chapter 2). The colonies inshore are also adapted to a wider annual and diurnal temperature range as the shallow waters rapidly warm and cool. The surrounding oceanic water moderates the Rim Reef and Terrace Reef temperature profile to a more favourable temperature range. The transplanted coral fragments would therefore be less temperature stressed with movement offshore to the Rim Reef, although experiencing a different temperature regime, which may cause initial stress. However, the occurrence of *M. mirabilis* colonies at the Terrace Reef indicates that neither seawater temperature or nutrient levels are a controlling factor on distribution. Finally, fish bite marks were sometimes seen on the transplanted *M. mirabilis* fragments, although occurrence was not seemingly correlated to a particular site. Fish grazing can differentially influence coral survival dependent on the resident fish populations (Neudecker, 1977; Neudecker, 1979; Glynn, 1988). Indeed, fish predation is believed to be the principle factor excluding *M. mirabilis* from shallow water (<13m) in the Florida Keys (Grottoli-Everett and Wellington, 1997). A further experiment is needed to assess the effect of grazing on *M. mirabilis* transplants, possibly by the use of caging exclusion experiments at the different reef zones.

The patchy survival rate of *M. mirabilis* fragments transplanted to the Outer Lagoon reef zone could not be readily related to any environmental conditions that differ between the replicate reef sites. The natural occurrence of *M. mirabilis* colonies is

similarly variable within the Outer Lagoon reef zone, although colonies were equally abundant at the study sites. The results indicate that specific conditions affecting the survival of *M. mirabilis* colonies and fragments may vary on a micro-environmental scale. Further experiments with additional Outer Lagoon sites are needed to assess whether the results are site specific or whether variation occurs throughout the reef zone. The survival of the *M. mirabilis* fragments at the Inner Lagoon indicates that this species is well adapted to the environmental conditions inshore. The niche specialisation is to the extent that the colonies do not survive well offshore at the environmental extreme at the Rim Reef indicating that the natural rarity of colonies offshore is not due to dispersal or necessarily recruitment failure but to unfavourable, species- specific environmental conditions.

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