Sexual Reproduction in the Caribbean Coral Genus *Mycetophyllia,* in La Parguera, Puerto Rico.

By

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ABSTRACT

Sexual reproduction is the most important process in the life history of corals because it provides the genetic variability to survive in changing environments. There is a lack of information about the reproductive biology in Caribbean corals. The aims of this study were to corroborate the available information about *Mycetophyllia ferox* and describe the sexual pattern, mode of development, the timing of the gametogenetic cycle, brooding season, reproductive traits and fecundity of *M. aliciae, M. lamarckiana* and *M. danaana*. Tissue samples of each species were collected for 14 months, fixed in Helly`s solution, decalcified in 10% HCl, dehydrated with EtOH, embedded in paraplast, and stained. Histological analysis revealed that *Mycetophyllia* species are simultaneous-hermaphrodites, with internal development of larvae, one gametogenetic cycle per year and different brooding seasons. Gametes maturation in *Mycetophyllia* species showed synchrony with the moon phase, water temperature rise, and light periods. *Mycetophyllia aliciae* showed significant higher mesenterial fecundity and *M. ferox* the lowest mesenterial fecundity.

RESUMEN

La reproducción sexual es el proceso más importante en el ciclo de vida de los corales, ya que provee variación genética para sobrevivir cambios ambientales. La información sobre la biología reproductiva de los corales en el Caribe es limitada. Los objetivos de este estudio fueron corroborar la información acerca de *Mycetophyllia ferox* y describir el patrón sexual, modo de desarrollo, duración del ciclo gametogenético, tiempo de planulación, estrategias reproductivas y fecundidad de *M. aliciae, M. lamarckiana* and *M. danaana.* Muestras de tejido de cada especie fueron colectadas por 14 meses, fijadas en solución Helly`s, decalcificadas en HCl 10%, deshidratadas en etanol, incrustadas en paraplasto y teñidas. El análisis histológico reveló que estas especies son hermafroditas-simultáneas, con desarrollo interno de larvas, tienen un ciclo gametogenético anual y diferentes periodos de planulación. La maduración de los gametos mostró sincronía con la fase lunar, aumento de temperatura y periodos de luz. *Mycetophyllia aliciae* mostró fecundidad mesenterial significativamente mayor y *M. ferox* menor fecundidad mesenterial.

DEDICATION

I dedicate this work to Mr. *Moisés R. Marcano López* for his support, encouragement and motivation during all the stages of this research, and for inspiring profound changes in my personal life during challenging moments in the search to become a better human being. Without his collaboration it would have been very difficult to develop and conclude this work. I also thank him for providing me the equipment that facilitated the preparation this document.

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I. INTRODUCTION

Coral reefs are diverse and complex tropical marine communities. They provide habitat, food, shelter and protection for a large diversity of marine organisms. At present, there is much ongoing research aimed at understanding the factors that contribute to and control coral community structure. Physical factors, such as storms, temperature, salinity, desiccation, as well as biological factors such as predation and competition, are important for reef development. If coral species have adapted and evolved in response to these factors, their life histories characteristics should reflect a selection of traits, adaptive to the various environments. Growth form and reproduction are two traits that can vary greatly between species, and thus are the result of natural selection (Szmant, 1986).

Like most of the world's reefs in the past years, Puerto Rican reefs have shown loss of living coral due to natural and anthropogenic causes. The rapid rates of human population growth in Puerto Rico have led to increased coastal development, dredging and sand extraction in near shore environments, deforestation for agriculture, and increased discharge of sewage and industrial waste. Some of the consequences associated with increased human pressure include high terrigenous sediment influx, increased nutrient levels, overfishing, and habitat modification. In addition, natural threats such as the island-wide mortality of the herbivorous sea urchin, *Diadema antillarum* in the 1980s, coral disease epizootics, bleaching, and storm damage have contributed to an overall reduction in coral abundance and cover, with corresponding increases in fleshy macroalgae (Morelock et al., 2001).

Knowledge of coral reproductive biology and the associated processes of dispersion and recruitment are essential prerequisites for ecological studies of coral populations, communities, and their dynamics. This in conjunction with a clear knowledge of the taxonomy, distribution, and the variability of different species, is essential for plans of population and community management of coral reef areas (Harrison and Wallace, 1990).

In corals, sexual reproduction consists of a sequence of events that include gametogenesis, release of the gametes from parental tissues, fertilization, and embryogenesis. Initial recruitment of larvae into a population is also part of the reproductive sequence because much of the selective pressure affecting the species will take place during these stages (Vance, 1973; Crisp, 1977). Local conditions such as annual temperature variation, lunar/tidal cycles, and variations in light-dark cycles, play a prominent role in determining the pattern of reproduction in scleractinians corals from different regions (Richmond, 1986). Regional shifts in the amplitude and periods of temperature cycles may produce observed shifts in the timing of reproduction among brooding and gamete-releasing corals, affecting reproductive periodicity and perhaps, intensity of fecundity in corals (Shlesinger and Loya, 1985; Stoddart and Black, 1985; Babcock et al., 1986). The timing, frequency, duration, intensity and spatial distribution of these events determine if the reproductive effort is successful.

Studies of coral reproduction have examined the cycles of reproduction in terms of the timing of gametogenesis, embryogenesis, and spawning in brooding and gamete-producing corals, and the factors that control these events (Harriot, 1983; Kojis and

Quinn, 1984; Fadlallah, 1985; Szmant-Froelich et al., 1985; Stoddart and Black, 1985; Chorensky and Peters, 1987; Szmant, 1986; Rinkevich and Loya, 1987). However, little is known about the reproductive patterns and characteristics of most coral species in the Caribbean, and most of the information is usually incomplete or conflictive (Vargas, 2002).

The genus *Mycetophyllia* is a Caribbean reef builder with five described species: *M. ferox* (Wells, 1973), *M. aliciae* (Wells, 1973), *M. lamarckiana* (Edwards and Haime, 1848), *M. danaana* (Edwards and Haime, 1848) and *M. reesi*. The only available reproductive study for this genus is for *M. ferox* (Szmant, 1985). There is a accute lack of information on fecundity and its variability among *Mycetophyllia* species.

This project aims to characterize the reproductive biology of three (*M. aliciae, M. lamarckiana* and *M. danaana*) of the four common species of *Mycetophyllia* in Puerto Rico and Caribbean coral reefs; using a year long analysis of histological samples and laboratory observations of the planulation cycles and their intensity. Other goals were to corroborate the results reported by Szmant-Froelich et al. (1985) for *M. ferox*, to assess the fecundity (per mesentery and polyp) for these species, and to compare all reproductive characteristics for species of *Mycetophyllia* and other brooding corals in the region. Results of this study enhanced our understanding of the reproductive biology and ecology of these organisms.

II. BACKGROUND

The oldest, most complex, resilient and diverse communities in the ocean are coral reefs. These reefs are located between 30º N and 30º S in three geographic regions: Indo-Pacific and Red Sea, with 85% of the world reefs and Western Atlantic with 8% in the Caribbean (Crossland, 1988) (Figure 1). According to Nybakken (1997), this distribution is influenced by parameters such as temperature 22-30 ºC (up welling areas), salinity 11-40 ‰ (river discharges), light (30-50 m depth), turbulence (abrasion and circulation), nutrients (oligothrophic areas), sedimentation (river discharges), substrate (hard and stable), and competition (macroalgae). There are 80 genera (600-700 zooxanthellae coral species) in the Indo-Pacific area, 27 genera (65-75 species) in the Atlantic-Caribbean, and 8 non-zooxanthellae species common to both provinces (Veron, 1995).

Corals are modular organisms, composed of repeated building blocks (modules) which are derived asexually by vegetative (iterative) growth. Modularity provides several adaptive advantages, such as: indeterminate growth (Connell, 1973; Hughes and Jackson, 1985; Sebens, 1987; and Hughes et al., 1992), longevities varying from a few months to several centuries (Hughes and Jackson, 1980; Cook, 1983; and Potts et al., 1985), iteroparous sexual reproduction (Charnov and Schaffer, 1973), high fecundity which may continue to rise indefinitely if growth is indeterminate (Jackson, 1979; Watkinson and White, 1985), morphological plasticity (Hall and Hughes 1996), some degree of functional or morphological differentiation (“polymorphism”) (Veron, 1993), and high survivorship.

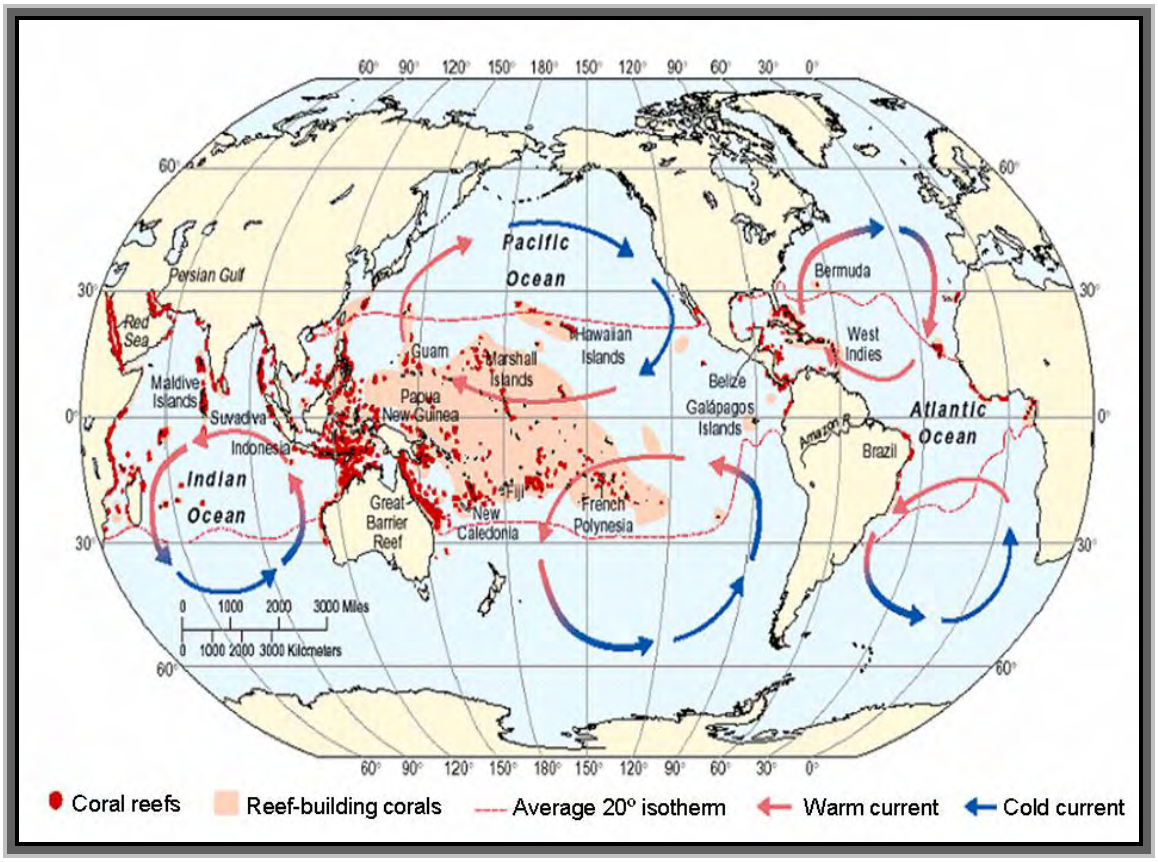


Figure 1. The global distribution of reef coral communities, including those that do not form structural reefs (Castro and Huber, 2005).

A. Importance of coral reefs

Coral reefs reduce wave action, allowing the development of seagrass in lagoons and mangroves along shoreline. These integrated ecosystems provide habitat, food, shelter and protection for many marine animals and plants. Coral reefs also protect coastal areas from erosion caused by waves and winds generated by tropical storms, reduce the influxes of river discharges, and filter nutrients and CO_2 from seawater and atmosphere. They constitute an important natural resource. Presently they are being

increasingly exploited in commercial activities such as fisheries, tourism and as source of biochemically active compounds for medical and pharmacological applications, raw building materials, ornamental objects and jewelry materials.

B. Reproductive biology of corals

B.1. Sexual vs. asexual reproduction

Reef building corals present a combination of sexual and asexual reproductive traits during their life-histories. Among the sexual strategies, corals can display hermaphroditism or gonochorism with no parental care, releasing the gametes into the water column (spawner or broadcaster) for external fertilization (Harrison et al., 1984a; Szmant, 1986; Harrison and Wallace, 1990). The resultant embryos live as plankton for a few weeks before settling. During this time there is high mortality but also long distance dispersion (Strathmann, 1985). There are also species with internal fertilization and development of embryos (brooders) (Harrison et al., 1984a; Szmant, 1986; Harrison and Wallace, 1990); larvae of these species are released into the environment ready for settling down and metamorphose into primary polyps, with higher survivorship, but with lower dispersal capabilities (Jackson, 1986). Another advantage of sexual strategies is to produce offspring with different genotypes, increasing the genetic variability of the population.

Among the asexual strategies, corals display: (i) budding with transverse or longitudinal fission to allow colony growth (Goreau and Goreau, 1959; Richmond,

1985b), (ii) fragmentation (Highsmith, 1982; Lirman, 2000), (iii) polyp bail-out, which results in a naked scleractinian polyp that can presumably form a skeleton (Sammarco, 1982), (iv) production of asexual larvae (Stoddart, 1983), from apomictic parthenogenesis inferred by Hartnoll (1977), (v) polyp balls, that consists of 1-30 polyps (Rosenm and Taylor, 1969), (vi) polyp expulsion, which results in the ejection of single polyps (Kramarsky-Winterrand et al., 1997), and (vii) production of gemmae, reported by Weil et al. (2000) as a novel strategy of asexual reproduction for *Diploria* and *Dendrogyra* in the Caribbean.. Asexual reproduction inevitably leads to some considerations of regenerative processes because, as Hoeksema (1989: 20) assert for fungiid corals, “Asexual reproduction is always the result of regeneration”. In fact, genes active in regeneration are likely also to be active in asexual reproduction (Grimmelikhuijzen, 2000). Asexual reproduction and regeneration produce clonal propagules (identical genetic replicates of adult colonies), which, if derived from fragments, have the advantages of large sizes and locally adapted genotypes (Richmond and Hunter, 1990). The rate of these strategies in the field may vary with habitat (Anthony and Svane, 1994), with density (Karlson et al., 1996), and with season (Chadwick-Furman and Spiegel, 2000).

B.2. Sexual patterns: gonochorism vs. hermaphroditism

Gonochoric species produce only female or male gametes within each colony during their lifetime. These species can be misinterpreted as sequential hermaphrodites and sequential cosexuals, because of the temporal separation in the development of each

sex and the timing of colony sampling (Harrison and Wallace, 1990). Hermaphroditic species produce both female and male gametes during their lifetime, and may be of two types: simultaneous, developing oocytes and spermatocytes at all times; or sequential, developing oocytes and spermatocytes at different times. Within sequential hermaphrodites, some species are sequential cosexual, in which the gametes develop sequentially during the same breeding season, or sequential alternating in which individuals change sex over their life or during different breeding seasons (protandrous: first male vs. protogynous: first female) (Policansky, 1982; Ghiselin, 1974).

Sequential hermaphroditism has not yet been demonstrated in scleractinian corals, although it has been recorded in other cnidarian groups (Policansky, 1982). Development of oocytes and spermatocytes can occur in the same polyp, intermingled within the same mesentery (syngonic), separately but adjacent in same mesentery (digonic) (favids and musids), in different mesenteries within the same polyp (pocilloporids and acroporids), or in different polyps within the same colony (*Astreoides calicularis* and *Porites astreoides*) (Loya, 1976; Loya and Rinkevich, 1979; Chornesky and Peters, 1987). Chornesky and Peters (1987) observed male, female and hermaphroditic polyps within hermaphroditic colonies (mixed breeding system) of *Porites astreoides* in the Caribbean (Table 1).

Hermaphroditism and gonochorism are conservative features of sexual reproduction, which have a phylogenetic basis (Harrison, 1985). Hermaphroditism predominates in the families Acroporiidae, Faviidae, Merulinidae, Musiidae, Pectiniidae, and Pocilloporidae. The families Agariciidae, Fungiidae, Caryophylliidae, Dendrophylliidae, and Flabellidae contain mainly gonochoric species (Harrison and

Wallace, 1990; Willis et al., 1985). There are exceptions to this generalization in coral sexuality, because *Astrangia, Montastraea, Porites, Scolymia* and Siderastrea show contrasting sexual patterns in the Pacific and the Caribbean (Table 1). Other genera, like *Montastraea,* and *Siderastrea* show both gonochorism and hermaphroditism (Harrison and Wallace, 1990). Szmant (1986) suggested that these reproductive differences may imply important differences in evolutionary selective pressures between the two zoogeographic regions. Among scleractinian corals, hermaphroditism is the dominant condition (Table 1 and Table 2) (Fadlallah, 1983; Harrison, 1985; Richmond and Hunter, 1990) and it could be considered the ancestral condition (Szmant, 1986).

Vargas (2002) studied sexual reproduction in the Caribbean genus *Diploria* in La Parguera, Puerto Rico during February 1999 to February 2002. The study included *D. strigosa, D. clivosa* and *D. labyrinthiformis.* These species are simultaneous-hermaphrodites with a single cycle of gametogenesis per year that starts with oogenesis a few months before spermatogenesis. *Diploria* species showed development and spawning of gametes in synchrony with full moon phase. A summary of her results is shown in Table 3.

Table 1. Summary of reproductive characteristics for Caribbean corals. Sexual patterns: H= hermaphroditic, G= gonochoric, M= mixed. Mode of development: S= spawn gametes, B=brooders, * = species with conflicting pattern or mode of development.

Species	Sexual mode	S/B	Source (locality)
Acropora cervicornis	H	S	Szmant-Froelich, 1984: Szmant, 1986 (Puerto Rico)
	H	S	Szmant, 1986 (Puerto Rico)
	H	S	Soong, 1991 (Panamá)
A. palmata	H	S	Szmant-Froelich, 1984: Szmant, 1986 (Puerto Rico)
	H	B	Peters, 1984 (St. Croix, Puerto Rico)
	H	S	Soong, 1991 (Panamá)
Agaricia agaricites	H	B	Duerden, 1902/ Van Moorsel, 1983/ (Caribbean)
	G		Peters, 1984 (St. Croix, Puerto Rico)
	M		Delvoye, 1982 (Cura cao)
A. crassa	?	B	Vaughan, 1910 (Florida)
A. fragilis	?	B	Mavor, 1915 (Bermuda, Caribbean)
A. humilis	H	B	Van Moorsel, 1983
	H	B	Van Moorsel, 1981 (Cura cao)
	M		Delvoye, 1982 (Cura cao)
Favia fragum	H	B	Duerden, 1902/ Vaughan, 1910
	H	B+S	Duerden, 1902 (Jamaica)
	?	B	Vaughan, 1908, 1910/ Matthai, 1919, 1923 (Tortugas)
	H	B	Lewis, 1974 (Barbados)
	H	B	Szmant-Froelich, 1984: Szmant, 1986 (Puerto Rico)
	H	B	Soong, 1991 (Panamá)
		B	Abe, 1937/ Motoda, 1939 (Palau)
Manicina areolata	?	B+S	Wilson, 1888 (Bahamas)
	?	B	Duerden, 1902 (Jamaica)
	?	B	Yonge, 1935/ Boschma, 1929 (Tortugas)
	H	B	Johnson, 1991, 1992 (Panamá)
Montastraea annularis	H	S	Szmant, 1991 (Puerto Rico)
	H	S	Soong, 1991 (Panamá)
	H	S	Wyers, et al., 1991 (Bermuda)
	H	S	Van Veghel, 1994 (Cura cao)
	H	S	Szmant et al., 1997 (Florida)
M. faveolata	H	S	Szmant, 1986 (PR)/ Szmant et al, 1997 (Florida)
	H	S	Van Veghel, 1994 (Cura cao)
	H	S	Soong, 1991 (Panamá)/Knowlton et al., 1997 (Cura cao)
M. franksi	H	S	Knowlton et al., 1997 (Cura cao)
	H	S	Szmant et al., 1997 (Florida)
M. cavernosa	G	S	Szmant-Froelich, 1984: Szmant, 1986 (Puerto Rico)
	G	S	Szmant, 1986 and 1991 (Puerto Rico)
	G	S	Wyers, et al., 1991 (Bermuda)
	G	S	Soong, 1991 (Panamá)
	G	S	Acosta and Zea, 1997 (Colombia)
	G	S	Hagman pers. Comm. with Dr.Weil (Flower Gardens Banks)

Table 1. Cont. Species	Sexual mode	S/B	Source (locality)
Porites astreoides	H	B	Szmant-Froelich, 1984 (Puerto Rico)
	H	B/S	Szmant, 1986 (PR)/Peters, 1984 (Puerto Rico)
	M	B	Chornesky and Peters, 1987 (Jamaica)
	M	B	Soong, 1991 (Panamá)
*P. porites**	G? some H	B	Tomascik and Sander, 1987 (Barbados)
		B	Goreau, 1981 (Jamaica)
	G	B	Soong, 1991 (Panamá)
	H	B	Vaughan, 1908, 1910 (Florida)
P. furcata	G	B	Soong, 1991(Panamá)
*Siderastrea radians**	H	B	Duerden, 1902 (Jamaica)
	G	B	Szmant-Froelich, 1984: Szmant, 1986 (Puerto Rico)
	G	B	Szmant, 1986 (Puerto Rico)
	G	B	Soong, 1991 (Panamá)
S. siderea	G	S	Szmant-Froelich, 1984: Szmant, 1986 (Puerto Rico)
	G	S	Szmant, 1986 (Puerto Rico)
	G	S	Soong, 1991 (Panamá)
	G	S	Guzman and Holts, 1993 (Panamá)
*Dendrogyra cylindrus**	G	S	Szmant-Froelich, 1984: Szmant, 1986 (Puerto Rico)
	6G	B	
	10H	S	
	3?		
	G	S?	Szmant, 1986 (Puerto Rico)
Diploria strigosa	H	B	Szmant-Froelich et al., 1983 (Puerto Rico)
	H	B	Peters, 1984 (St.Croix)
	H	S	Szmant, 1986 (Puerto Rico)
	H	S	Wyers, 1985 (Bermuda)
	H	S	Soong, 1991 (Panamá)
	H	S	Wyers et al., 1991 (Bermuda)
	H	S	Gittings et al., 1992 (East and West Flower Garden Bank)
	H	S	Steiner, 1995 (Puerto Rico)
	H	S	Vargas, 2002 (Puerto Rico)
D. clivosa	H	S	Soong, 1991, 1993 (Panamá)
	H	S	Vargas, 2002 (Puerto Rico)
D. labyrinthiformis	?	S	Wyers et al., 1991 (Bermuda)
	H	B	Duerden, 1902 (Jamaica)
	H	S	Vargas, 2002 (Puerto Rico)
Mycetophyllia ferox	H	B	Szmant, 1986 (Puerto Rico)
	H	B	Morales, this study (Puerto Rico)
M. aliciae	H	B	Morales, this study (Puerto Rico)
M. lamarckiana	H	B	Morales, this study (Puerto Rico)
M. danaana	H	B	Morales, this study (Puerto Rico)

Table 2. Zoogeographical comparisons of scleractinian coral reproductive characteristics. Information of the Pacific regions and Red Sea is from Richmond and Hunter (1990), and the Caribbean information is from Table 1.

Localities	Red Sea	Pacific region	Caribbean
# species studied per locality	15	209	22
Gonochoric	0	42	5
Hermaphroditic	15	143	11
Mixed	0	24	6
Spawners	11	173	9
Brooders	3	27	11
Confused	1	9	2

Pacific regions = Hawaii, Central Pacific, and Great Barrier Reef. Confused = incomplete data or different reported data for the same species.

Table 3. Summary of reproductive characteristic of *Diploria* species. Sexual pattern: S-H = Simultaneous-Hermaphrodite. Mode of development: S= spawn gametes (broadcasters). Lunar phase: d= days; AFM= after full moon. From Vargas, 2002.

Species	*D. strigosa*	*D. clivosa*	*D. labyrinthiformis*
Breeding season	Single cycle of gametogenesis/year.	Single cycle of gametogenesis/year.	Single cycle of gametogenesis/year.
Sexual pattern	S-H	S-H	S-H
Mode of development	S	S	S
Oogenesis duration	Nov-Jan 10-11 months	Nov-Jan 10-11 months	June-May 10-11 months
Spermatogenesis duration	May-June 3-4 months	Jul-Aug 3-4 months	Jan-April 4-5 months
Spawning	Jul-Aug	Jul-Aug	April or May
Lunar phase	6-10 d AFM	6-10 d AFM	4 d AFM
Fecundity	3-4 oocytes/mesentery 8-9 spermatocytes/ mesentery	4 oocytes/mesentery 6 spermatocytes/me-sentery	5-6 oocytes/mesentery 7 spermatocytes/me-sentery

The reproductive biology characteristics in *Mycetophyllia* are only know for one of the five species (Szmant, 1986: Table 4; Figure 2). Based on histological analysis and laboratory observations, she reported that *M. ferox* is a simultaneous- hermaphroditic and broods larvae during the winter (Table 4; Figure 2). The species has mostly platy colonies, early sexual maturation, low recruitment rates, high juvenile mortality, and only one gametogenic cycle per year with several planulation events (Szmant, 1986). Also spermatocyte production outnumbers oocyte production.

Table 4. Summary of reproductive patterns and estimates of average annual egg volume production per cm^2 surface area for *Mycetophyllia ferox* in Puerto Rico [a] = Maximum diameter measured in histological sections: [b] = Estimated from species descriptions in Smith (1971): [c] = Mature oocytes at a time; many immature ones present also: ♂= spermatogenesis: ♀= oogenesis. From Szmant, 1986.

Species	*M. ferox*
Sexual mode	Hermaphrodite
Egg size µm [a]	300
Gametogenesis	♂ Nov.-Dec. ♀ Apr.-Aug.
Spawning Season	Dec.-Jan.; planulation Feb.-Mar.
Brooding Season	January-March
Number eggs/mesentery	2-4
Number mesenteries/polyps	12-24
Number polips/cm^2 [b]	2
Number of reproduction cycles/year	1-2
Total egg volume mm^3/cm/yr	1.51
Type of development [c]	Brooder

B.3. Mode of development: spawning vs. brooding

Coral species which broadcast spawn, outnumber brooders in the Pacific region and the Red Sea by the ratio of 168:37 (Table 2). Spawning is usually associated with higher fecundity, large colonies, late sexual maturation, low recruitment rates, low adult mortality, and predictable habitats (Van Moorsel, 1983). However, approximately 60% of the scleractinian Caribbean corals are reported to be brooders (Vermeij et al., 2003) (Figure 2). Brooding is generally associated with small colony size, high rates of adult mortality, early maturation, production of many small offspring over a longer breeding period, and is believed to be favored in disturbed environments (Stearns, 1992; Ayre et al., 1997; Van Moorsel, 1983). Exceptions can be found in the Indo-Pacific, where brooding species form relatively large colonies (Kojis, 1986b). Shlesinger et al. (1998) concluded, that "At present there seems to be no universal relationship between coral size, polyp size and or egg size and mode of reproduction".

C. Fecundity

The fecundity of clonal animals and plants is determined by the number of gravid modules (polyps, zooids, shoots, ramets), their reproductive output (Hall and Hughes, 1996); and varies over the lifetime of the coral, with include pre-reproductive, adolescent, mature and some times senescent stages (Harrison and Wallace, 1990). Sexual maturity and fecundity depend on the interaction between colony size and polyp age, and are not simply a function of either one (Kojis and Quinn, 1985).

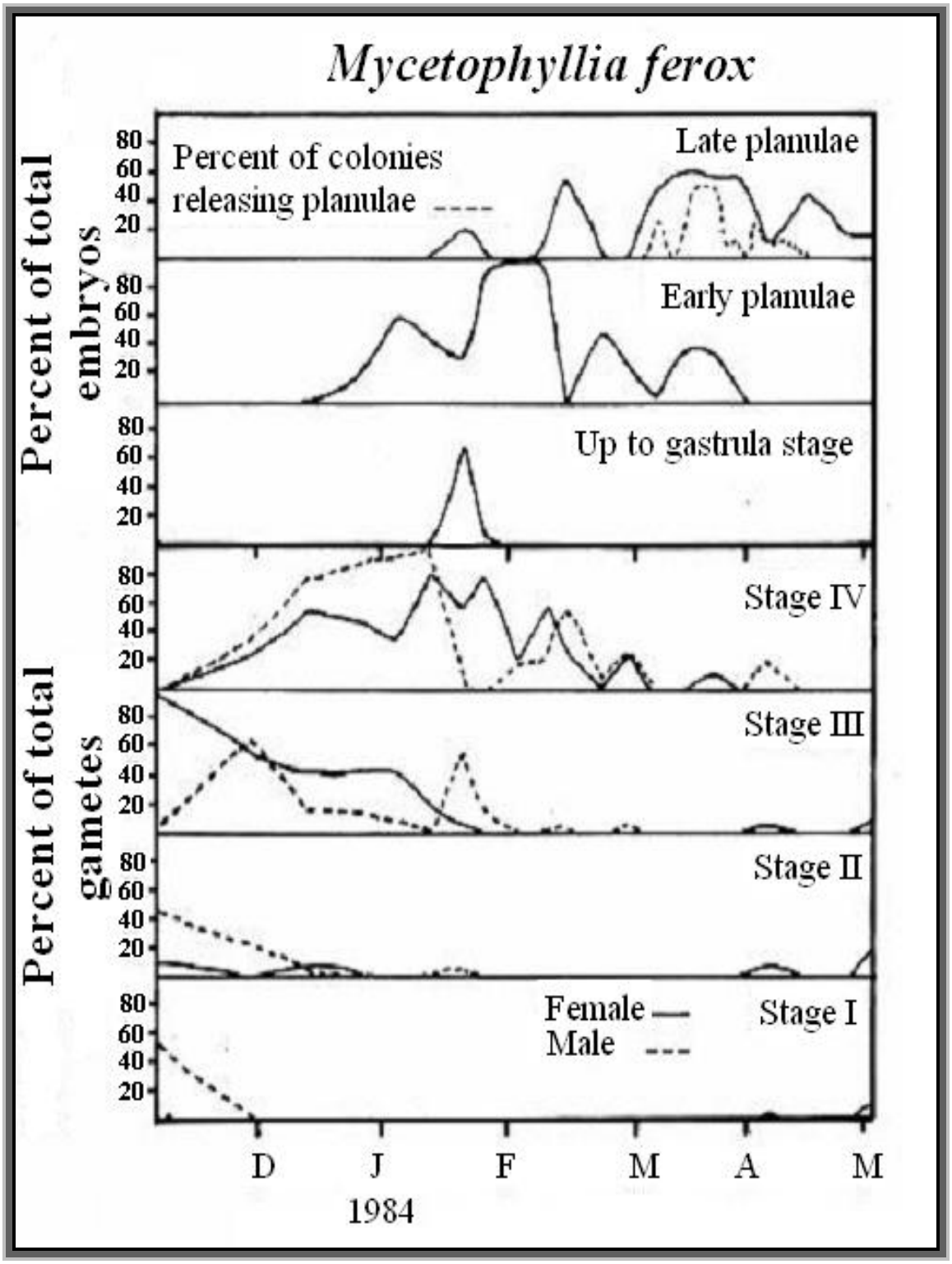


Figure 2. Representative reproductive cycle for *M. ferox*: Period shown is from November 1983 to May 1984, during which weekly samples were taken. Stages I-IV are categories of increasing gametocyte maturity with IV being ripe. The upper box includes the percent of colonies releasing planulae during an in situ planulation study (Szmant, 1986).

In *Goniastrea favulus*, colony size determined whether gametogenesis occurred, but colony age influenced both size at first reproduction and polyp fecundity. Thus, the minimum breeding size was larger for undivided young colonies of *G. favulus* than for fragmented older ones, and if two colonies were of the same size but different ages, the older colony was more fecund (Kojis and Quinn, 1985). In a similar study, Szmant-Froelich (1985) found that colony size affected the ability of *Montastraea annularis* colonies to reproduce. Coral fecundity normally increases with age as a result of increase in the number of polyps, an increase of polyp fecundity, or both. Also, polyp fecundity may rise through an increase in the number of egg-bearing mesenteries (*Enallopsammia willeyi:* Gardiner, 1900), or an increase in the number of eggs per mesentery.

Fecundity varies spatially within reproductively mature colonies, among colonies of a population, and between populations of some coral species. In species with localized regions of growth, polyps close to actively growing areas are sterile (Rinkevich and Loya, 1987). Polyps in the "post-sterile" zone may have reduced fecundity or produce only testes; however the length of sterile zone varies (Chorensky, 1989; Harrison and Wallace, 1990; Soong and Lang, 1992). Beyond these regions, the number of oocytes per polyp is relatively constant within each colony, including axial polyps, which are not actively extending (Oliver, 1984; Wallace, 1985). These sterile zones and regions of lowered fecundity must be considered when samples are collected for studies of gametogenesis, or when total colony fecundity is estimated (Harrison and Wallace, 1990).

A variety of human perturbations cause sub-lethal stress in corals and reduce fecundity. Because a colony typically must be a minimum size to reproduce sexually

(Hall and Hughes, 1996; Kapela and Lasker, 1999; Smith and Hughes, 1999), its output from sexual reproduction can be affected by breakage of the skeleton (Zakai et al., 2000) or other damage (Oren et al., 2001). Typically such perturbations have negative effects (Kalafatiae, 1997; Schierwater and Hadrys, 1998), at least in part because resources are diverted for repair (Oren et al., 2001).

D. Gametogenesis

Because cnidarians have diploblastic tissue-level of organization, scleractinians have no true reproductive organs. Development of oocytes and spermatocytes takes place within mesenteries, enveloped by mesoglea and gastrodermis (Campbell, 1974; Chapman, 1974). The origin of germ cells is still controversial (interstitial vs. amoebocytes), but, germ cells in anthozoans are considered to be of endodermal origin. More research is needed to establish if germ cells are derived from interstitial cells, dedifferentiated amebocytes or mesogleal cells (Fadlallah, 1983).

Oogonia, or primary oocytes, migrate into the mesoglea of the mesenteries from the overlying gastrodermis (Szmant-Froelich et al., 1985) and develop within the mesoglea lamellae of the mesenteries for internal or external fertilization (Szmant-Froelich et al., 1980; Delvoye, 1982; Fadlallah and Pearse, 1982a). On the other hand, spermatocytes first appear as a thin opaque structure that grows to contain discrete sacs, which are separated from one another by a thin layer of mesoglea within mesenteries (Szmant-Froelich et al., 1980). Szmant-Froelich et al. (1985) classified oocytes, spermatocytes and embryo development into stages (Table 5) to describe the process of gametogenesis in corals. Gametocytes initiate development at different times (Figure 2),

but usually mature simultaneously through a population for synchronized spawning; if delayed it could result in “split-spawning” (Willis et al., 1985). Mature stages in oocytes and spermatocytes are recognizable by their color, shape, size and location (Kojis and Quinn, 1981; Harrison et al., 1984; Babcock et al., 1986; Szmant, 1986). Oocytes tend to be more brightly colored (red, orange and pink) than spermatocytes, due to the pigments in maturing oocytes (Harrison and Wallace, 1990).

The hermaphroditic genus *Mycetophyllia,* like other members of the family Musiidae, develops oocytes and spermatocytes separately, but adjacent in the same mesentery (digonic pattern; Harrison, 1985). In this family, the head and midpiece of the sperms are pear-shaped or ovoid. This sperm type is found in some hermaphroditic corals, and may be characteristic of hermaphroditic corals in general (Harrison, 1985 and 1988b). In Puerto Rico, Szmant (1985) found no mesenteries with gametocytes in *M. ferox* colonies smaller than 100cm². Based on histological observations, this author found that *M. ferox* had five to ten times more large spermatocytes than oocytes. This would appear to be extremely wasteful if self-fertilization was common, and rather implies an intense effort to out-cross, similar to that of trees that produce enormous quantities of pollen (Baker, 1959; Williams, 1975).

Table 5. Criteria for classification of gametocytes and embryos into developmental stages following Szmant-Froehlich et al. (1985).

Stages	Oocytes	Spermaries	Embryos
O	No ova in mesentery.	No spermaries in mesentery.	No planulae in coelenteron.
I	Enlarged interstitial cells with large nuclei in mesoglea of mesentery.	Small clusters of interstitial cells near or entering mesoglea.	Same size and staining properties as eggs, but free from mesentery. includes development up to the two layered gastrula stage.
II	Accumulation of small amount of cytoplasm around nuclei.	Clusters of spermatocytes with distinct spermary boundary; large nuclei.	Early planula; mesoglea present. oral pore and coelenteron form. No stains red.
III	Oocytes of variable size; Main period of vitellogenesis.	Spermatocytes smaller with smaller nuclei; number of cells within spermary much larger.	Mesenteries forming as invaginations of mesoglea and endoderm.
IV	Oocytes full size with indented nucleus; stains dark red with H-H.	Spermatocytes with little cytoplasm; tails not evident.	Well developed septa; mature planula.
V	----------	Spermatozoa with tails; ready to spawn.	----------

E. Brooding season

Sexual reproduction in corals may occur yearly, seasonally, monthly, or not at all. Brooding species tend to breed and release planulae throughout an extended periods or year-round (Harrison and Wallace, 1990). No clear trends are apparent with regard to the breeding seasons of brooding corals. The season and duration of breeding and subsequent planula release varies greatly among species (Shlesinger and Loya, 1985; Kojis, 1986a). Breeding seasons and periods of planula release tend to be shorter at higher latitudes, and occur over extended periods, or year-round nearer the Equator. For example, *Acropora*

palifera breeds only once in spring at Heron Island (30^0S), but year-round at Lizard Island (14^0S) and in reefs near Lae (7^0S) (Kojis, 1986b). Similar latitudinal trends are apparent in *Seriatopora hystrix* and *Stylophora pistillata*, which breed and release planulae seasonally in the Red Sea (29^0N) and GBR (Loya, 1976; Rinkevich and Loya, 1987; Loya, 1983b; Shlesinger and Loya, 1985), but release planulae year-round in Palau (Atoda, 1947b, 1951c). *Stylophora pistillata* breeds and releases planulae during the same months but in opposite seasons in the northern (Red Sea) and southern (GBR) hemispheres (Loya, 1983b). In the Caribbean, *Mycetophyllia ferox* broods during winter (January-April; 3-months), *Siderastrea radians* releases planulae year-round, *Porites astreoides* broods from January to September (9 months), and *Favia fragum* has lunar gametogenetic and embryogenic cycles year long (Szmant-Froelich et al. 1985; Szmant, 1986).

F. Planula development and behavior

Brooded planulae develop more slowly that non-brooded larvae, and may take weeks or months to complete its development. Zooxanthellae are often incorporated into the planula tissues during development, and may enter through the ectoderm near the oral pore (Szmant-Froelich et al., 1985; Chornesky and Peters, 1987). Mesenteries form around strands of mesoglea, and the endodermal layer becomes increasingly ordered (Szmant-Froelish et al., 1985). Further development leads to a mature planula form with a differentiated epidermis, mesoglea, gastrodermis, stomodaeum, mesenteries, and coelenteron. Sometimes the stomodaeum, coelenteron and mesenteries form after the planulae are released (Edmondson, 1929). The morphology, structure and behavior of

mature coral planulae have been extensively studied in brooding species (Duerden, 1902; Atoda, 1953; Harrigan, 1972; Fadlallah, 1983). Brooded planulae are generally larger than externally developed planulae. The smallest planulae recorded are produced by the broadcast-spawning coral *Astrangia danae* and are 75 µm in diameter; the largest are brooded by *Mycetophyllia ferox* and grow to 3 mm in diameter (Szmant-Froelich, 1984; Szmant, 1986).

G. Environmental factors regulating sexual reproduction

Environmental conditions may influence sexual processes as proximate factors, which provide cues to synchronize reproductive cycles, and as ultimate factors, which exert selective pressure on the synchrony and timing of breeding (Giese and Pearse, 1974; Clark, 1979). Among the proximate factors are sea temperature, daylight, salinity, food, moonlight, tidal cycles and daily light/dark cycles (Harrison and Wallace, 1980). These factors may act differently to coordinate each stage of gamete development, or different factors may operate at each phase (Korringa, 1947; Clark, 1979). Lunar and daily light cycles may act as guide signals to stimulate spawning after a specific period of darkness during the first appropriate moon phase after the maturation of gametes (Willis et al., 1985; Babcock et al., 1986). Other environmental factors, such as seasonal changes in day length, water quality and food, may also influence reproductive cycle in corals (Delvoye, 1982; Fadlallah, 1982; Fadlallah and Pearse, 1982a; Kojis, 1986b). The ultimate factors controlling maturation and spawning of gametes include: photoperiod, temporal reproductive partitioning, environmental constraints and predation pressure (Shlesinger and Loya, 1985). These factors are advantageous because they increase the

probability of fertilization, reduce gamete wastage, and enhance the potential for outcrossing in self-fertile hermaphrodites (Harrison and Wallace, 1990).

H. Lunar periodicity and planula release

The lunar timing and duration of planula-release periods varies widely among brooding species, with some species not exhibiting lunar periodicity of planula release (Fadlallah, 1983; Motoda, 1939; Jells, 1980; Shlesinger and Loya, 1985; Kojis, 1986a; Wright, 1986). Periods of planula release are generally less clearly delineated than breeding periods. Many brooders release planulae over a range of lunar phases, with shorter periods of peak release. Colonies of *Favia fragun* from Puerto Rico released planula between 6 and 15 days after the new moon, with peak release from days 8 to 11 (Szmant-Froelich et al., 1985). Seasonal changes in the lunar phase of planula release have been reported in *Pocillopora damicornis* from the northern Great Barrier Reef (Marshall and Stephenson, 1933; Harriot, 1983).

Planula release also occurs predominantly at night in populations of *Agaricia* spp., *F. fragun*, *P. damicornis*, *P. astreoides* and *Stylophora pistillata* (Vaughan, 1908; Rinkevich and Loya, 1987; Lewis, 1974; Harriot, 1983; Van Moorsel, 1983; Szmant-Froelich et al., 1985; Kojis, 1986a). Colonies of *F. fragun* in Barbados released approximately equal numbers of planulae during the day and night before the first quarter moon phase. Planula release in *Balanophyllia elegans* and *P. damicornis* in Hawaii occurred at various times, with apparent differences between day and night (Harrigan, 1972; Fadlallah and Pearse, 1982a).

I. Characteristics of the species of *Mycetophyllia*

Taxonomy: Kingdom: **Animalia**, Phylum: **Cnidaria**, Class: **Anthozoa**, Order: **Scleractinia**, Family: **Mussidae**, Genus: ***Mycetophyllia.***

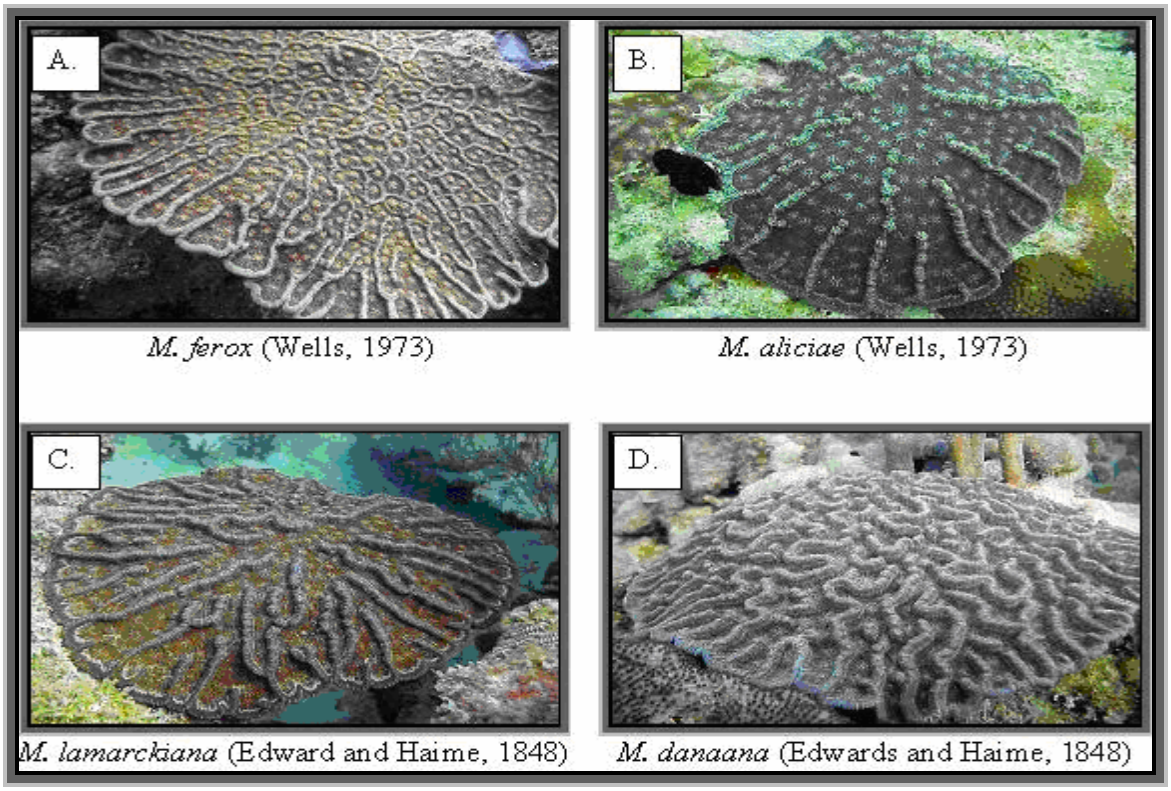


Figure 3. Species of *Mycetophyllia* sampled for histological analysis of gametogenesis and embryogenesis. *M. ferox,* described by Wells, 1973 (A); *M. aliciae,* described by Wells, 1973 (B); *M. lamarckiana,* described by Edward and Haime, 1848 (C); *M danaana,* described by Edward and Haime, 1848 (D). Photos provided by Dr. Ernesto Weil.

I.1. *Mycetophyllia ferox*

Corallum an expanding lamina weakly attached (subpedunculate), irregularly circular in outline in large colonies. Calicular surface even, undulant. Corallum marginally thin, thickening toward the area of fixation to 4-5 cm. Undersurface with only faint traces of epitheca, costate as in other species. Growth with narrow uniserial rows of centers separated by low, narrow collines over entire upper surface. Valleys shallow,

scarcely more than 10 mm wide except at forking. Collines continuous, interconnecting. Septal and septocostal marginal dentations tall, acute, and minutely spinulose, about 1 mm apart, four or five per septum, the innermost one taller than the rest. The distinctive characters of this species are the continuous, low, interconnecting submeandroid collines enclosing the narrow valleys with single rows of polyps (Figure 3-A).

South Florida, Bahamas and Caribbean. Tends to inhabit shaded areas of midrange reefs and along walls. Most common between 30-70 meters. Polyps retracted during the day.

I.2. *Mycetophyllia aliciae*

Corallum commonly circular lamina, weakly attached centrally to substrate. Undersurface with thin shreds of epitheca overlying radiating, thin, equal, toothed costae. Growth with di- or triserial series of centers separated by low, narrow, straight, radiating collines. Collines at origins about 3 cm apart, spreading to 6 or 8 cm before terminal forking introduces intervening short collines. Septa 10-24 per center, but not quite extending to the axis, which is free of columellar structure. Septa equal in thickness, and equal to, as thick as, or thicker than, the interspaces. Septa and septocostae six or seven per cm. Septal and septocostal marginal dentations short and conical, 10 or 11 per cm (Figure 3-B).

South Florida, Bahamas and Caribbean. Tends to inhabit shaded areas of midrange reefs and along walls. Most common between 10-40 meters. Polyps retracted during the day.

I.3. ***Mycetophyllia lamarckiana***

Medium size (up to 12 cm in diameter), weakly attached, often turbinate colonies with a nearly circular calicular surface. The central, founder polyp is surrounded by a ring of 7-10 polyps budded circumorally. Subsequently, each marginal polyp forms a chain of polyps intramurally, a colline forming between each chain. A chain may split, in which case a new colline is added. Continuous valleys, 10-20 mm wide, all radiate from the central polyp. Septa coarsely dentate. Direct lamellar linkage. Rudimentary columella (Figure 3-C).

South Florida, Bahamas and Caribbean. Tend to inhabit shaded areas of moderate and deeper reefs, often on ledges and along walls. Most common between 10-30 meters. Polyps retracted during the day.

I.4. ***Mycetophyllia danaana***

Massive, weakly attached, irregularly-shaped, meandroid colonies up to 35 cm in diameter. Founder polyp (calice) usually not apparent. Sinuous valleys long and continuous, 8-17 mm wide. Valleys usually deep (10-15 mm) but may be shallow (4 mm). Coarsely dentate septa. Collines sometimes discontinuous or hydnophoroid. Direct lamellar linkage. Columella rudimentary or absent (Figure 3-D).

South Florida, Bahamas and Caribbean. Tend to inhabit shaded areas of shallow to moderately deep reefs. Most common between 10-25 meters. Polyps retracted during the day.

III. OBJECTIVES

A. Main objectives

1. To characterize the reproductive patterns (hermaphroditic or gonochoric) and mode of development (brood or broadcast) of three species of *Mycetophyllia* in Puerto Rico.
2. To characterize the gametogenetic cycles over time of the three species of *Mycetophyllia,* in terms of the onset, phases, and duration of each cycle, the distribution of gametes and larvae in the mesenteries using histological procedures.
3. To assess fecundity (mesentery and polyp) and its variability across *M. aliciae, M. ferox, M. lamarckiana* and *M.danaana.*
4. To characterize the variability in sexual reproduction among the four common species of *Mycetophyllia* in Puerto Rico by determining differences in gametogenesis, brooding timing, fecundity and oocyte sizes.

B. Proposed questions

1: What is the sexual pattern and mode of development of the other three species of *Mycetophyllia (M. aliciae, M. danaana* and *M. lamarckiana)*?

2: What is the timing of the gametogenetic cycle and the breeding season for these three species of *Mycetophyllia* in Puerto Rico?

3: Are there any differences in the reproductive traits among the four species of *Mycetophyllia* in Puerto Rico?

C. Null and alternative hypotheses

H_{01}: There are no differences in the mode of development among the *Mycetophyllia* species.

H_{11}: At least one species shows a different mode of development.

H_{02}: There are no differences in the sexual pattern among the four species of *Mycetophyllia.*

H_{12}: At least one species shows a different sexual pattern.

H_{03}: There are no differences in the timing of gametogenesis among the species.

H_{13}: At least one species shows differences in the timing of gametogenetic cycle.

H_{04}: There are no differences in fecundity among the *Mycetophyllia* species.

H_{14}: At least one species has a significantly different fecundity level.

IV. MATERIALS AND METHODS

A. Study sites

Tissue samples were collected during 2000, 2001 and 2004 from the following reefs in La Parguera, southwest coast of Puerto Rico: San Cristobal, Cayo Enrique, Caracoles, Media Luna, Beril, Pináculos and Turrumote. These localities are at varying distances from the coast (Figure 4). Surface temperatures ranged from 26.0˚C to 31.0˚C from March 16, 2000 to May 5, 2001. San Cristobal, is located at 17° 56.497` N and 67° 04.497` W, is a long and well developed fringing reef with a mid slope down to 15m deep. Media Luna, is located at 17° 56.08` N and 67° 02.864` W, is a fringing reef, with an extended platform and a drop to 20m. The platform is covered mainly with soft corals (gorgonians). The front area is characterized by a slope with different species of corals that ends in a sandy area at a depth of 20m. The Buoy Site (El Beril), is located at 17° 53.291` N and 66° 59.884` W, is a submerged bank reef (18m-40m) on the edge of the insular platform, 8 km from the south coast of Puerto Rico. Caracoles, is located at 17º 57.643` N and 67º 02.126` W, is a shallow fringing reef that run from northeast to southwest direction, with narrow platform and reach 15m deep. Enrique, is located at 17º 55.480` N and 67º04.335` W, is a fringing reef and a drop to 15m. Turrumote, is located at 17º 56.097` N and 67º 01.130` W, is a fringing reef and a drop to 20m. Pináculos, is located at 17º 56.065` N and 66º59.100` W, is a patch reef and a drop to 20m (Table 6). The reef forms, spurs and grooves and has a diverse community. These reefs were selected because of the proximity to the Isla Magueyes Marine Laboratories of Department of Marine Sciences of the University of Puerto Rico-Mayagüez Campus and

the presence of the four species of *Mycetophyllia*. Table 7 shows the number of specimens per locality and the total number of specimens collected for each species.

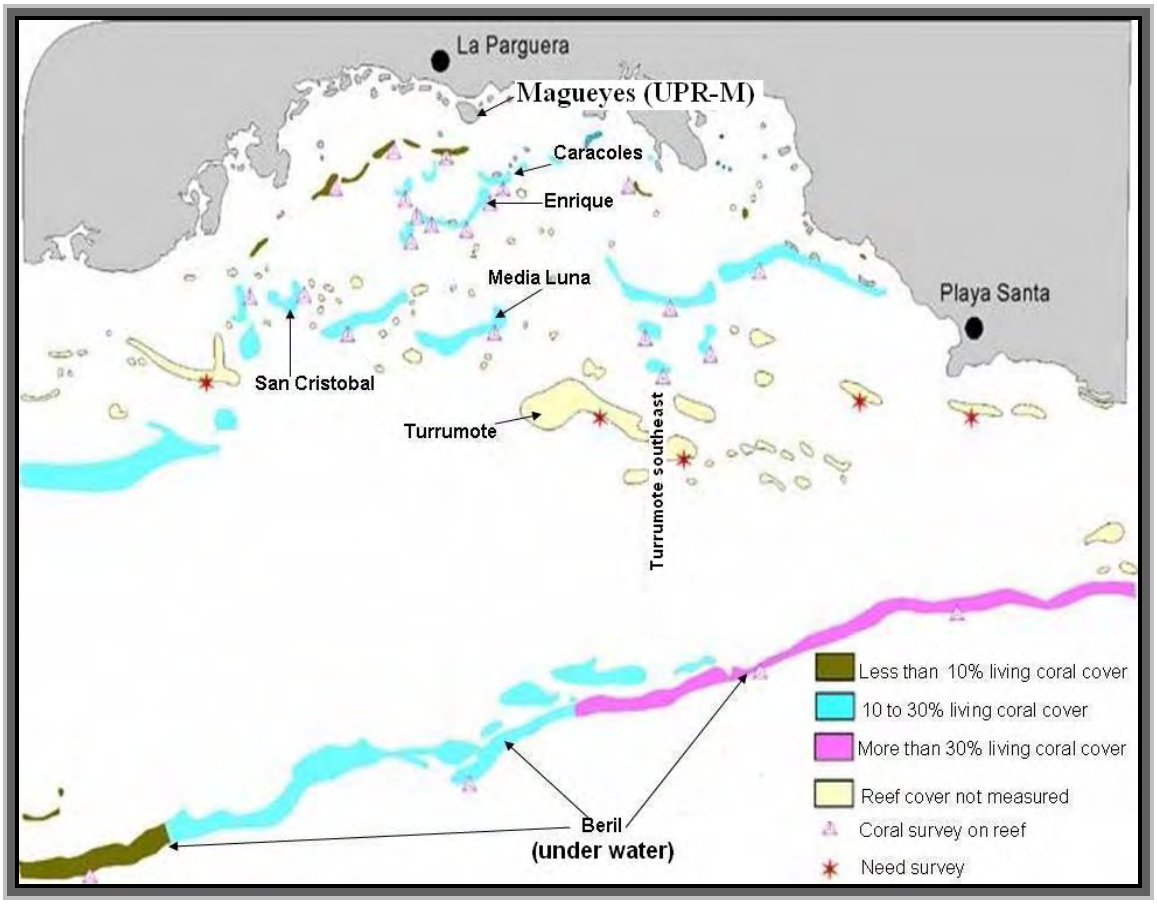


Figure 4. Reefs of La Parguera in the southwest coast of Puerto Rico from which the samples were collected during years 2000-01 and 2004. (UPR-M) = University of Puerto Rico-Mayagüez Campus. Diagram from Morelock et al., 2001.

B. Field collection

From 1999 to 2002, and November 2004, Tissue samples were collected every month for 14 months for 24 species of Caribbean corals in Puerto Rico by Weil and Urreiztieta. Surface area [max. length x min. length] of colonies was measured to corroborate that they surpassed 100cm², the minimum reproductive size reported (Szmant,

1986). Using a whole saw bit (3.18-3.49 cm) and a hammer, one core (~2.5 cm in diameter) in the central area of at least 5 colonies of each species was removed every month. Cores were placed in individual, labeled plastic bags, with seawater and transported to the laboratory. Tissue samples were preserved, and sectioned for histological analysis. This project used histological tissue samples from this collection to characterize the sexual reproductive patterns of the other three species in the genus *Mycetophyllia (M. aliciae, M. lamarckiana* and *M. danaana)*, common in Puerto Rican waters, for which there was no reproductive information.

Table 6. Characteristics of sampled localities during 2000-2001 and November 2004.

Localities	classification	Latitudes N	Longitudes W	Depth m
Cayo Enrique	Fringing	17º 55.480`	67º 04.335`	15
Caracoles	Fringing	17º 57.643`	67º 02.126`	15
Media Luna	Fringing	17º 56.08`	67º 02.864`	20
Beril	Bank	17º 53.291`	66º 59.884`	18-40
San Cristobal	Fringing	17º 56.497`	67º 04.497`	15
Turrumote	Fringing	17º 56.097`	67º 01.130`	20
Pináculos	Patch	17º 56.065`	66º 59.100`	20

Table 7. Number of specimens *Mycetophyllia* species collected per locality.

Localities	*M. ferox*	*M. lamarckiana*	*M. danaana*	*M. aliciae*
Cayo Enrique	4	0	3	2
Caracoles	1	0	3	5
Media Luna	9	26	14	48
Beril	14	22	16	11
San Cristobal	26	11	1	0
Turrumote	21	29	18	17
Pináculos	0	0	4	4
Turrumote (2004)	5	0	0	7
Total of specimens collected	80	88	59	94

C. Laboratory processing

C.1. Sample fixation and decalcification

To preserve tissue samples each core was labeled with a piece of Mylar paper and wrapped in cheesecloth. Cores were placed in Zenker Formalin (Helly's solution) (APPENDIX A) for 16-24 h, and rinsed in tap water for 24 h. Rinsed cores were placed in glass containers with 10% hydrochloric acid (HCl) solution for decalcification (APPENDIX B). Solutions were changed twice every day until decalcification was completed. Samples were checked for $CaCO_3$ residue using the 5% ammonium oxalate test: 1 ml of 5% ammonium oxalate was added to five ml of the HCl solution in the containers and allowed to stand for five minutes; if a white precipitation occurred, the decalcification was not completed. After complete decalcification, tissues were rinsed with distilled water and cleaned of endolithic algae and other organisms embedded in the skeleton. Finally, the samples were stored in 70% ethanol (APPENDIX C).

C.2. Embedding and cutting

Preserved samples were dehydrated in different concentrations of ethanol (70% and 95%) and isopropanol solution (Tissue Dry), cleaned in xylene solution (Tissue Clear III) and placed in the tissue processor to fill the tissues with paraplast. Samples were embedded in paraplast (Tissue Prep, melting point 56.0-57.0°C) using the Tissue Tek Tec apparatus, and placed in a freezing plate (Tissue Tek) at – 3.0°C until the paraffin solidified. Samples were stored in the freezer for at least 24 h before sectioning. Longitudinal and cross sections (7-10 µm) were obtained from the tissue samples using a rotary microtome (Leitz 1512). Strip sections were placed in a warm bath (Boekel) at 48.0-50.0°C, to let the tissue strips stretch. The strips were lifted up on a slide and placed

on a slide warmer (Precision) at ≈ 48.0°C for about 1-2 h. Slides with tissue samples were then stored in boxes at room temperature for at least 24 h to allow the tissue strip to stick to glass slide and dry well before staining.

C.3. Staining

Tissue samples were stained with Heidenhain`s Aniline-Blue method (Animal Histology Procedures: Coolidge and Howard 1979) to examine the maturation stages of gametocytes and embryos. Tissue samples were deparaffinized with xylene solution, then slowly hydrated with distilled water using 100%-95%-70% ethanol solutions. Slides were stained in preheated (56.0°C) Azocarmine B solution, rinsed in tap water, followed by distilled water for a few minutes. Afterward, samples were soaked in aniline-alcohol, mordant in phosphotungstic acid and stained with aniline-blue solution to differentiate cytoplasm and connective tissues. Finally, samples were dehydrated through 70%-95%-100% ethanol solution, cleared with xylene solution and mounted in slides with cytoseal solution to seal the tissue in the slides. Tissue samples were examined with an Olympus BX 40 compound microscope and photographed with a pixera digital camera. The sexual pattern and gametogenetic cycle of each species were determined by observing and identifying the stages of gametocyte development through the year of collection. Gametocyte maturation sequence for each species was characterized following criteria by Szmant- Froelich et al. (1985) (Table 5). Oocyte diameters were measured with a Sigma Scan Program calibrated to millimeters which, then were converted to micrometers.

D. Fecundity

Mesenterial fecundity (number of eggs + number of larvae/mesentery) was estimated by counting the number of oocytes in stage IV and/or larvae in each mesentery and dividing this by the total average number of mesenteries in a polyp with eggs and/or larvae. Polyp fecundity of each colony was determined by counting the total number of eggs in stage IV and larvae (Total number eggs + Total number larvae) per polyp in a tissue sample. Usually, each polyp has an average 24 mesenteries (Szmant, 1986). A maximum and minimum diameter (μm) of oocyte and larvae was measured and averaged for each species of *Mycetophyllia* to compare the egg sizes among the different stages (I-IV) within each species and across species.

E. Environmental variables

Records of temperatures, lunar cycles and day/night light for the years 2000 and 2001 were used to verify if the timing of gametogenetic cycles and brooding events were correlated with these variables. Temperature data was from NOAA/NASA AVHRR Oceans Pathfinder dataset. The lunar phases were documented using a calendar that shows the days of each phase. The day/night light period was documented using a sun and moon calendar that shows the timing of sun rise and set and the moon rise and set.

F. Statistical analysis

The statistical analysis was performed with Infostat and SigmaStat programs. A nonparametric one-way ANOVA test (Kruskal-Wallis) was used to test the differences in

larvae and oocyte diameters (μm) by stage (I-IV) within each species, and among the species, to test differences in mesenterial and polyp fecundity among the four species of *Mycetophyllia*. This test was used because data failed the normality test and variances were not equal ($p < 0.050$). One-way analysis of variance (ANOVA) was used to test the differences in *M. ferox* oocyte diameters, and in larvae diameters among the four species, after data passed the normality and an equal variance tests ($p > 0.050$).

V. RESULTS

A. Sexual patterns and mode of development

Tissue slides confirmed that *M. ferox* is a simultaneous-hermaphrodite with the onset of oogenesis three months before the onset of spermatogenesis (Table 8; Figure 5-A). *Mycetophyllia aliciae* and *M. lamarckiana* are simultaneous-hermaphrodites with simultaneous onset of oogenesis and spermatogenesis (Table 8; Figure 5-B, C). *Mycetophyllia danaana* is a simultaneous-hermaphrodite with the onset of oogenesis and spermatogenesis at different times, maturating spermatocytes before oocytes during the same breeding season (Table 8; Figure 5-D). All species have one oogenesis cycle and one spermatogenesis cycle, except *M. danaana* which may be had two spermatogenesis cycles (Table 8). In all species, oocytes and spermatocytes developed adjacent to one another in the same mesentery with syngonic and digonic arrangement, and in separate mesenteries (digonic). All species had internal embryogenesis (brooders), with larvae developing over different periods and brooded at different times (Table 8; Figure 6-A, B, C and D).

Brooding was not observed in the field, however, but larvae production of the four species was observed in the laboratory during 2000 and 2001 in La Parguera, Puerto Rico (Weil pers. comm.) (Table 9). These observations were repeated with *M. ferox* and *M. lamarckiana* from January to April 2005; *M. ferox* brooded from March to April, 2005 and *M. lamarckiana* in April, 2005.

Table 8. Summary of gametogenetic timing and reproductive characteristics for *Mycetophyllia* species. ♀C = oogenesis cycle, ♂C = spermatogenesis cycle. ♀♂-S = simultaneous hermaphrodite. Moon synchrony = gamete maturation during full moon phase.

Mycetophyllia	***ferox***	***aliciae***	***lamarckiana***	***danaana***
Oogenesis	June - Jan (7-months)	June - May (11-months)	Aug - Jul (11-months)	May - May (12-months)
Spermatogenesis	Sep - Jan (4-months)	June - May (11-months)	Aug - Jul (11-months)	May - Mar / Feb - Jul (10 / 5-months ?)
Brooding season	Dec - May (6-months)	Mar - Apr (2-months?)	Aug - May (9-months ?)	Mar - Apr (during year ?)
Gametogenic cycles per year	1-♀C 1-♂C	1-♀C 1-♂C	1-♀C 1-♂C	1-♀C 2-♂C ?
Sexual mode	♀♂-S	♀♂-S	♀♂-S	♀♂-S
Type of development	Brooder	Brooder	Brooder	Brooder
Moon synchrony	Full moon	First quart	First quart	Full moon

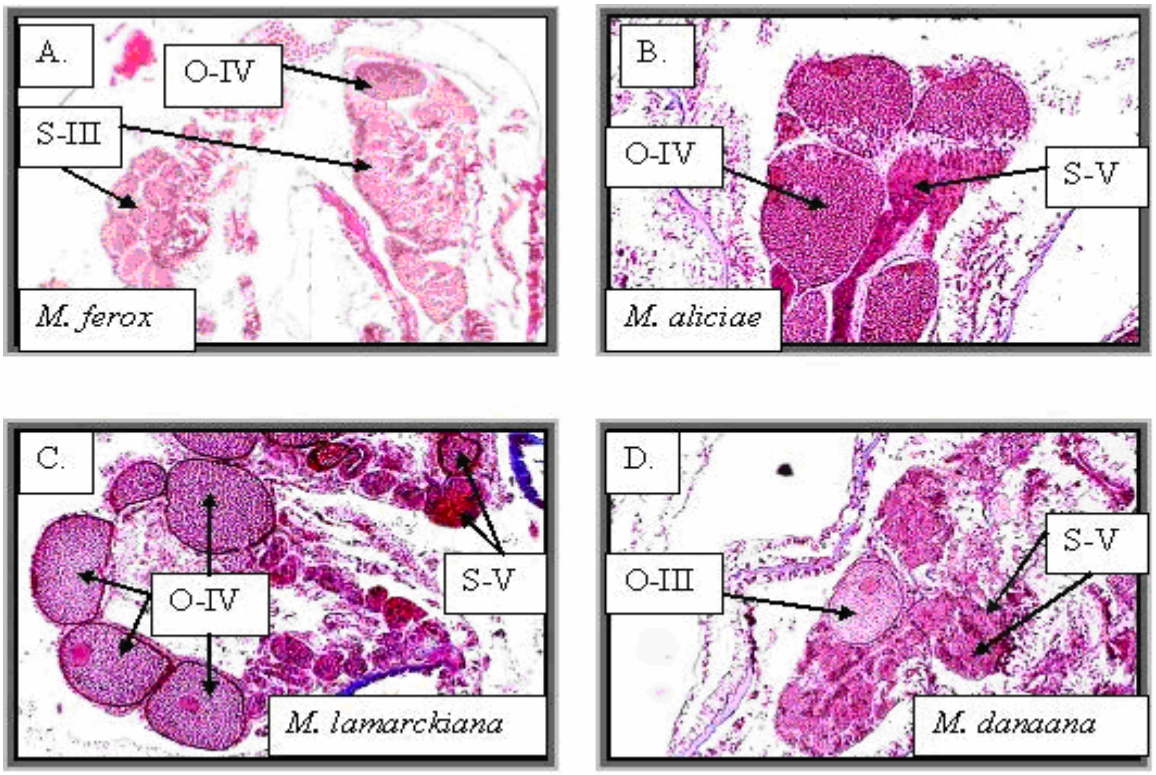


Figure 5. Tissue sections of *M. ferox* (A), *M. aliciae* (B), *M. lamarckiana* (C), and *M. danaana* (D) (magnification 4x), showing their hermaphroditic condition, the different stages of oocytes (O) and spermatocytes (S), and their distribution across mesenteries. Mature stages: IV in oocytes and V in spermatocytes.

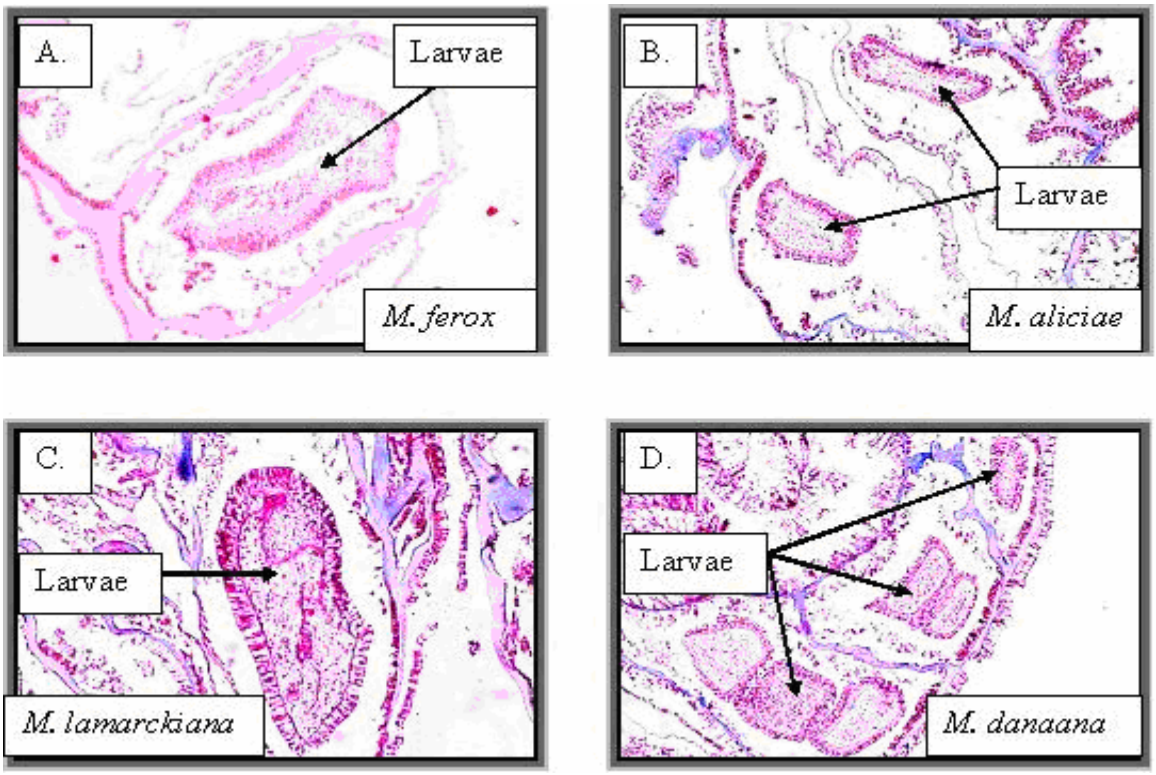


Figure 6. Tissue sections showing internal development of larvae in *M. ferox* (A), *M. aliciae* (B), *M. lamarckiana* (C), and *M. danaana* (D) (magnification 4x).

Table 9. Laboratory observations of planula release in *Mycetophyllia* in Puerto Rico, indicating that planula release is not correlated with moon cycle.

***Mycetophyllia* species**	**Date brood**	**Days after full moon**	**Days after new moon**	**Larvae/ colony**
M. danaana	02/03/2001	13	27	8
M. lamarckiana	02/11/2000	7	5	26
M. aliciae	02/11/2000	7	5	8
M. ferox	02/28/2000	29	9	10
	02/02/2001	12	26	2
	02/03/2001	13	27	12
	02/10/2001	2	4	10
	02/14/2001	6	8	6
	02/17/2001	9	11	18
	02/20/2001	12	14	5
	02/21/2001	13	15	2
	02/23/2001	15	0	4

Data provided by Dr. Ernesto Weil

B. Gametogenesis and embryogenesis

In scleractinian corals gamete development occurs within the mesenteries and the process was analyzed and classified following criteria by Szmant-Froelich et al. (1985) (Table 5). Analysis of longitudinal sections revealed, that oocytes in all species of *Mycetophyllia* developed (a) surrounded or intermingled with spermatocytes (syngonic), (b) parallel with spermatocytes through the mesentery (digonic), (c) at the bottom of mesenteries with spermatocytes located towards the coelenteron (digonic), or (d) in different mesenteries (digonic). These patterns are characteristic of all *Mycetophyllia* species (Figure 7).

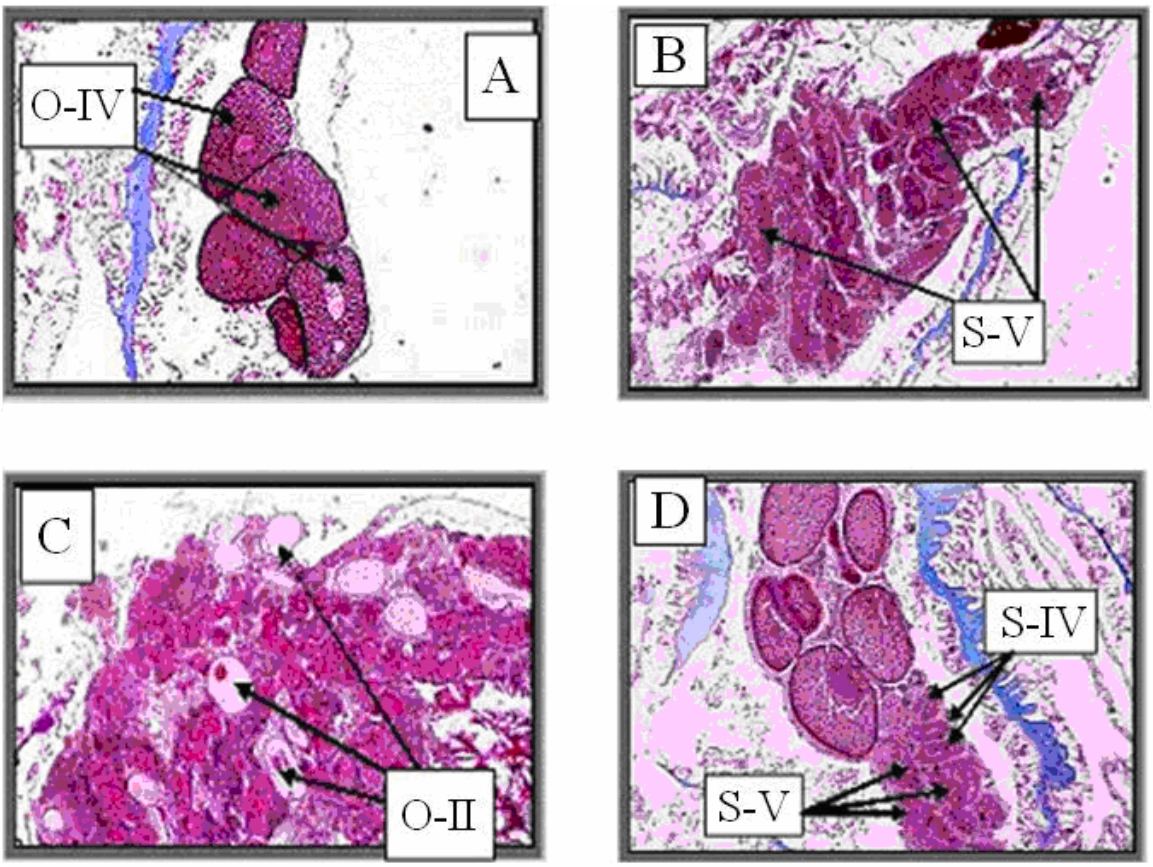


Figure 7. Characteristic gamete arrangement in *Mycetophyllia* species. Female mesentery, A; Male mesentery, B; Syngonic mesentery, C; and Digonic mesentery, D.

Embryogenesis takes place within mesenteries, but larvae develops unbounded to the mesoglea lining. The development of embryos in *M. ferox, M. aliciae, M. lamarckiana* and *M. danaana* was classified into four stages following criteria by Szmant-Froelich et al. (1985) (Table 5). Stage I embryos, which includes development up to the 2-layered gastrula resembles oocytes in size. Stage II represents an early planulae stage with mesoglea, surrounded by cilia and larger than oocytes in stage IV. Larvae in stage III show invaginations of mesoglea and endoderm; stage IV larvae are pear shaped or elongated and have well-developed septa with zooxanthellae in the gastrodermis.

B.1. *M. ferox* oogenesis

Histological results corroborated that this species has a single cycle of gametogenesis per year, lasting approximately 8 months, with the onset of oogenesis occurring in June (Figure 8 and Figure 13). Also, this species has oocytes in different stages of development within the mesenteries at the same time. Development from one stage to the next takes moderate periods of time, with progressive maturation of the oocytes from stage I to IV taking 8 months. Stage I had a duration of 4 months (June to October); primary oocytes had an average diameter of about 7.05 (± 1.44) µm, an enlarged nucleolus with little cytoplasm surrounding the nucleus, and a thick cytoplasmic membrane (Figure 8-A). Oocytes in stage I have significantly smaller average diameter than the rest of the stages ($p < 0.0001$) (Table 10; Figure 9). In July, the oocytes differentiated into stage II, as evidenced by the increase in cytoplasm around the nucleous, larger average diameter of 10.85 (± 1.26) µm (Figure 8-B), and staining bluish. By September, 80% of sampled colonies were in stage II (Figure 13). At this stage, the

oocytes stained pink, showed thinner cytoplasmic membrane and granulose cytoplasm due to vitellogenesis. Stage III, oocytes were observed in September and were significantly 14.48 (± 1.68) µm larger (ANOVA, $p < 0.0001$) in average diameter than stage II oocytes. Stage IV developed by November, and December the oocyte enlarged to 18.83 (± 1.60) µm, significantly larger (ANOVA, $p < 0.0001$), the coloration changed to dark red, and the cytoplasm showed a more granulose appearance. By December, 80% of the colonies sampled displayed stage IV oocytes. At this stage oocytes were significantly larger than at previous stages (ANOVA, $p < 0.0001$) (Table 10; Figure 9). Samples from November 2004 agreed with this observations (Figure 14). This study demonstrated that this species has oocytes at different developmental stages within the mesenteries at the same time.

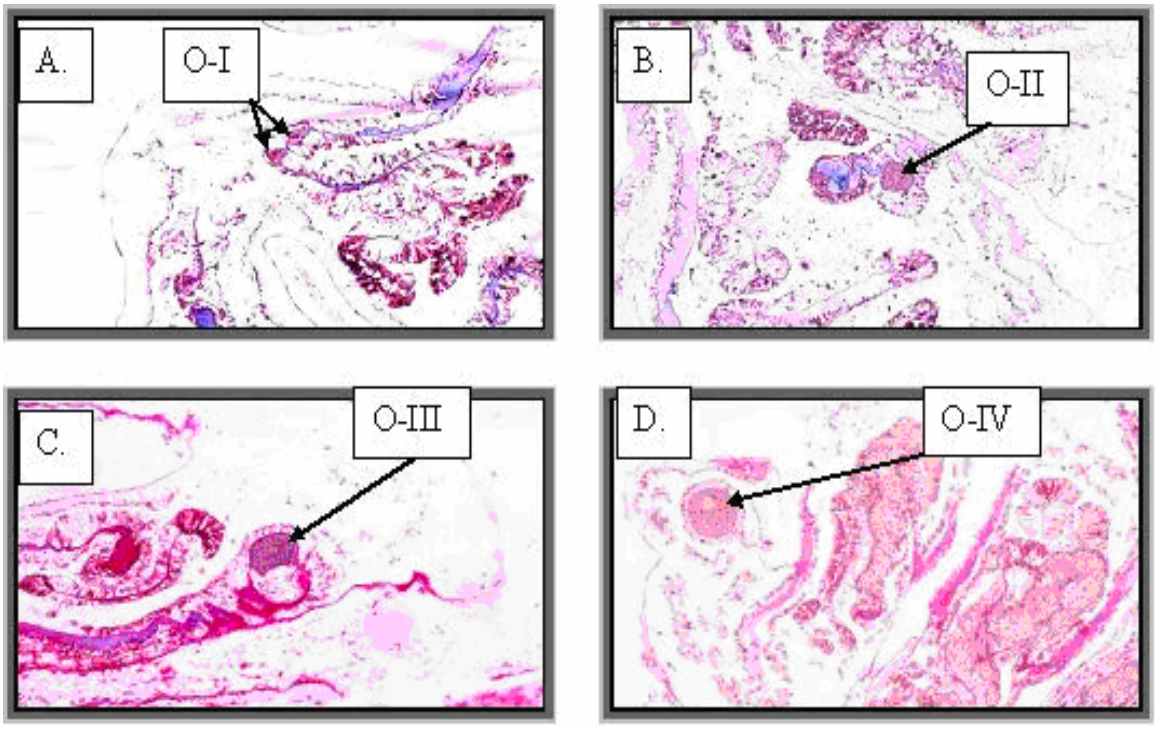


Figure 8. Different stages of oocyte (O) development (O-I to O-IV) showing pattern of spatial arrangement of oocytes and spermatocytes in *M. ferox*. C. shows hermaphrodite mesentery and D. shows male mesentery and female mesentery.

Table 10. One-way ANOVA analysis of variance and LSD Fisher test for oocyte diameters in *M. ferox*. Different capital letters indicate significant differences ($p < 0.0001$).

Variable	N	R^2	R^2 Aj	CV
Diameter	69	0.88	0.88	11.89

Analysis of variance (SC type I)

F.V.	SC	gl	CM	F	p-valor
Model	1100.66	3	366.89	165.29	<0.0001
Stages	1100.66	3	366.89	165.29	<0.0001
Error	144.28	65	2.22		
Total	1244.94	68			

Test: LSD Fisher Alfa: =0.05 DMS: =1.02368

Error: 2.2196 gl: 65

Stages	Media	n				
Stage I	7.05	15	A			
Stage II	10.85	22		B		
Stage III	14.48	19			C	
Stage IV	18.83	13				D

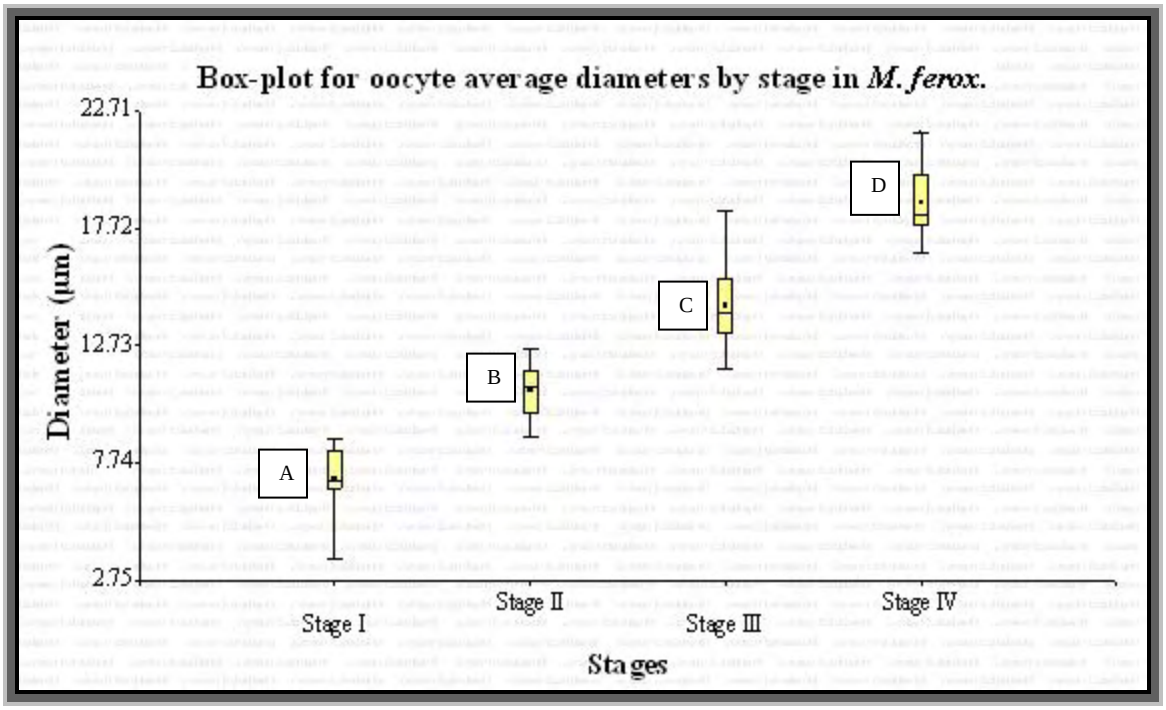


Figure 9. Box-plot for oocyte average diameters by stage in *M. ferox*. Y axis diameters measurements in (µm), X axis developmental stages I-IV. Different capital letters (A-D) indicate significant difference among the stages.

B.2. *M. ferox* spermatogenesis

Histological results corroborated that this species has one cycle of spermatogenesis per year, lasting 5 months approximately, with the onset in September, three months after oogenesis (Figure 10 and Figure 13). The time lapse between stage changes was approximately a month, with progressive maturation from stage I to V in approximately 5 months (Figure 13). The different stages of spermatogenesis were classified following Szmant-Froelich et al. (1985) criteria (Table 5). The first recognizable stage I consists of small light blue-gray groups of cells with a large nuclei accumulating adjacent to the mesoglea in the mesenteries (Figure 10-A). Stage II tends to be difficult to discern from stage I because they look like enlarged interstitial cells and most of the time they are in clusters both mixed with mesoglea or oocytes (Figure 10-A). In stage III a membrane formed around clusters of spermatocyte cells, which migrated inside the mesentery around oocytes or near the oocyte towards the coelenteron (Figure 10-B and Figure 13). In December, spermatocytes changed to stage IV in which cells became greater in number, smaller in size and stained in a light red color (Figure 10-C-F). Spermatocytes in stage V stained in a red wine color because of presence of chromatin in their nucleus and the decrease of cytoplasm around the nucleus. Forty percent of colonies had stage V spermatocytes in January (Figure 13).

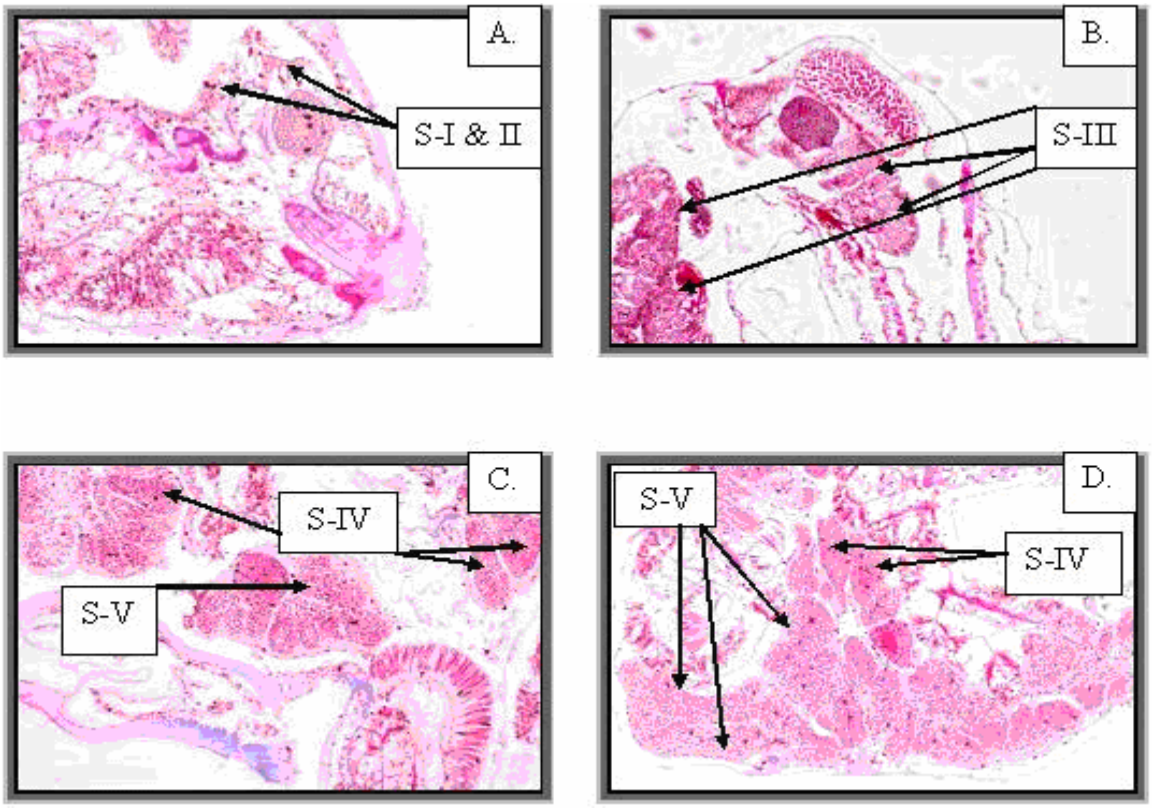


Figure 10. Spermatocyte (S) developmental stages (S-I to S-V) and arrangement within the mesentery in *M. ferox* (magnification 4x). A. and B. oocytes at the bottom of mesenteries, with spermatocytes located towards the coelenteron; C. and D. oocyte surrounded or intermingled with spermatocytes.

B.3. *M. ferox* embryogenesis

Analysis of longitudinal sections of tissue samples (Figure 11 and Figure 13) showed that this species produce larvae from December to April, with only one extended period of planula development (Figure 13). At the onset of embryogenesis, some mesenteries showed embryos and others showed spermatocytes in stage V. By December, 60% of colonies showed planulae in stage I with similar size as oocytes (average diameter of 9.8 ± (1.67) µm). In January 100% of larvae developed into stage II with an average diameter of 57.85 (± 14.68) µm (Figure 11 and Figure 13). There was significant difference in the average diameter between stages I and II ($p < 0.0001$) (Table 11; Figure

12). In February, stage III embryos were observed with invaginations of mesoglea to form mesenteries and an average diameter of 83.33 (± 13.62) µm (Figure 11-C). In March, 80% of sampled colonies had larvae in stage IV with well developed septa and an average diameter of 85.2 (± 24.68) µm. Stages III and IV were significantly bigger (Kruskal Wallis, $p < 0.0001$) than stages I and II. There were not significant differences between stages I and II (Table 11; Figure 12). Samples obtained in November 2004 did not show planulae. This species developed one larvae per mesentery at a time and was the only species in which the number of larvae was correlated to the number of oocytes produced.

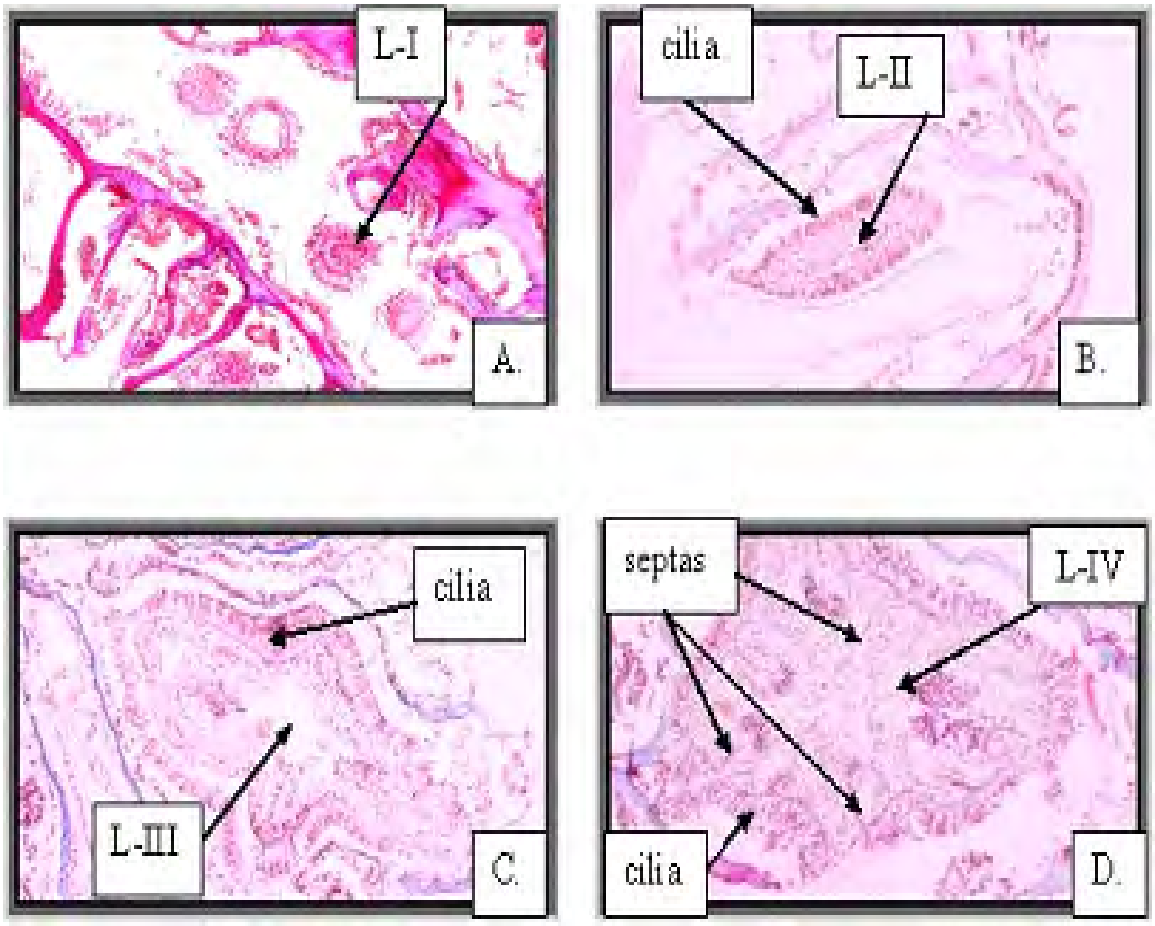


Figure 11. Larvae (L) developmental stages (L-I to L-IV) within mesenteries in *M. ferox* (Magnification in 4x). A stage I with similar characteristics as oocytes but free from mesenteries membrane, B stage II larvae with mesoglea surrounded by cilia, C stage III larger planula with invaginations of mesoglea and endoderm, D larvae with well developed septa.

Table 11. Non parametric one-way ANOVA test (Kruskal Wallis) for larvae diameters in *M. ferox*. Different capital letters indicates significant differences ($p < 0.0001$).

Variable	Stage	N	Media	D.E.	Median	Average range	
Diameter	I	3	24.85	1.89	25.26	2.00	gl= 3
Diameter	II	22	57.85	14.68	56.21	18.27	C= 1.00
Diameter	III	23	83.33	13.62	82.82	38.96	H= 27.95
Diameter	IV	8	85.19	24.68	78.91	36.50	p< 0.0001

Trat.	Ranks		
Stage I	2.00	A	
Stage II	18.27	A	
Stage IV	36.50		B
Stage III	38.96		B

Figure 12. Box-plot for larvae average diameters by stage in *M. ferox*. Y axis diameter measurements in (µm), X axis developmental stages I-IV. Different capital letters (A-C) indicates significant difference among the stages.

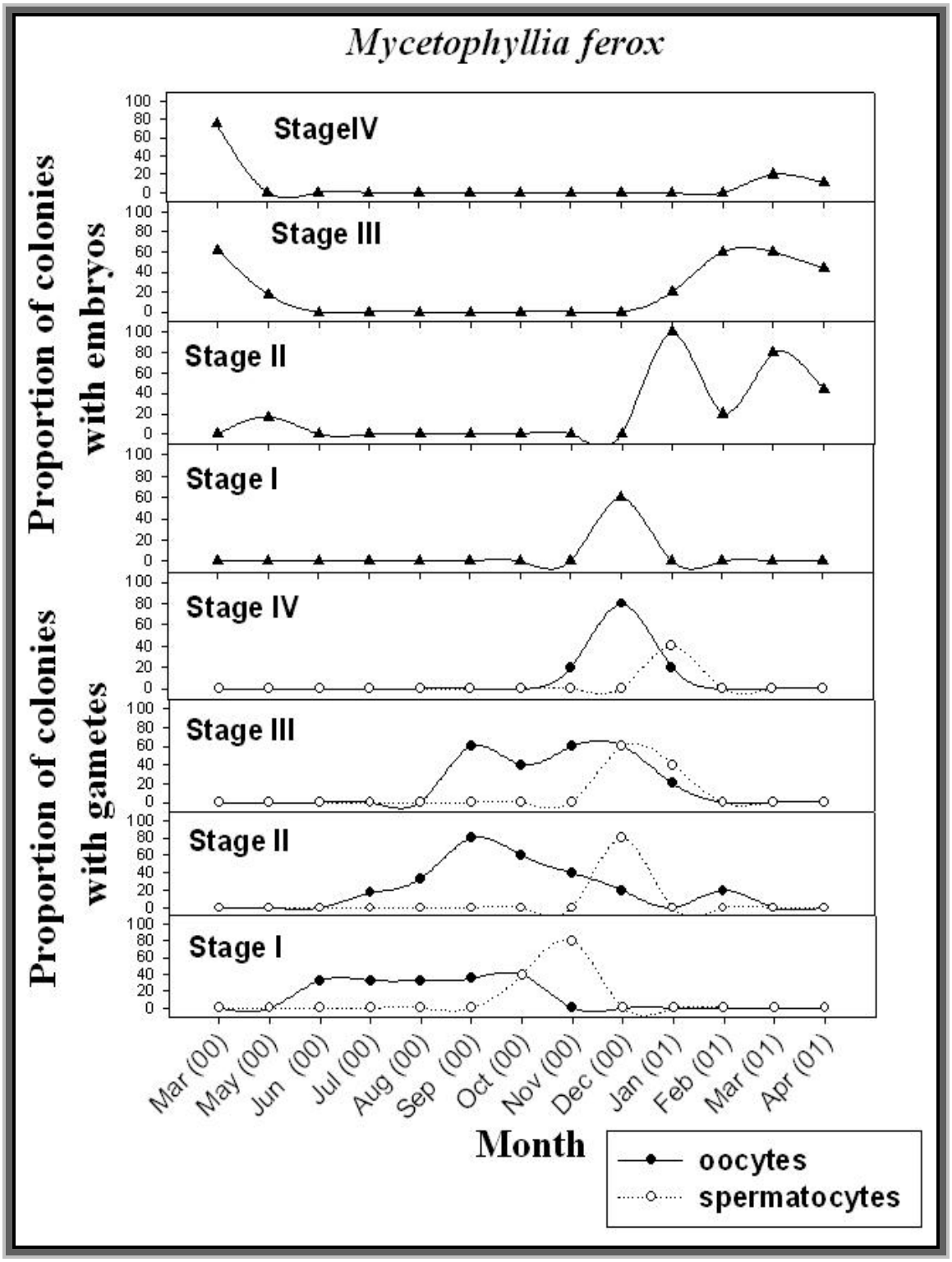


Figure 13. Representative reproductive cycle for *M. ferox*: Period shown is from March 2000 to April 2001, when monthly samples were taken. Stages I-IV are categories of increasing gamete and embryos maturity, with IV being ripe. Y-axis is the percent of colonies with gametes and embryos in each stage of development.

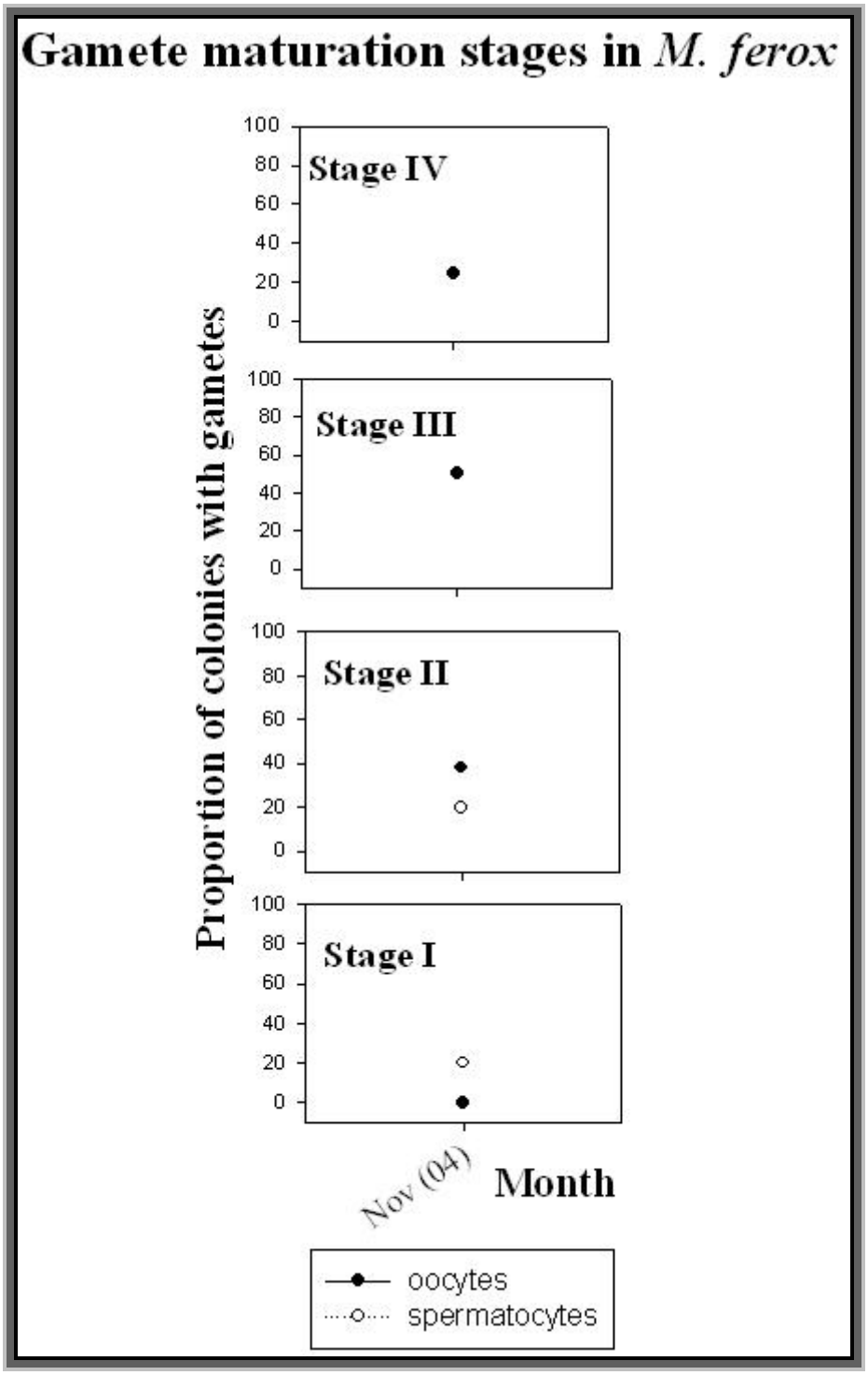


Figure 14. Gamete maturation stages for *M. ferox.* Period shown is for November 2004. Stages I-IV are categories of increasing gamete maturity, with IV being ripe. Y-axis is the percent of colonies with gametes in each stage of development.

B.4. *M. aliciae* oogenesis

This species had a single cycle of oogenesis per year and had different stages of development within the mesenteries at all times. The onset of oogenesis was in June and remained active for 11 months (Figure 15 and Figure 19). In this species, stages I and II oocytes were present for 10 months. Stage I began in June, and these primary oocytes were 5.78 (± 2.86) µm in average diameter (Figure 15). In October, 100% of sampled colonies were in stage I (Figure 19). Stage II oocytes were observed in June, and in December 100% of sampled colonies were in stage II, which lasted 10 months (Figure 19).

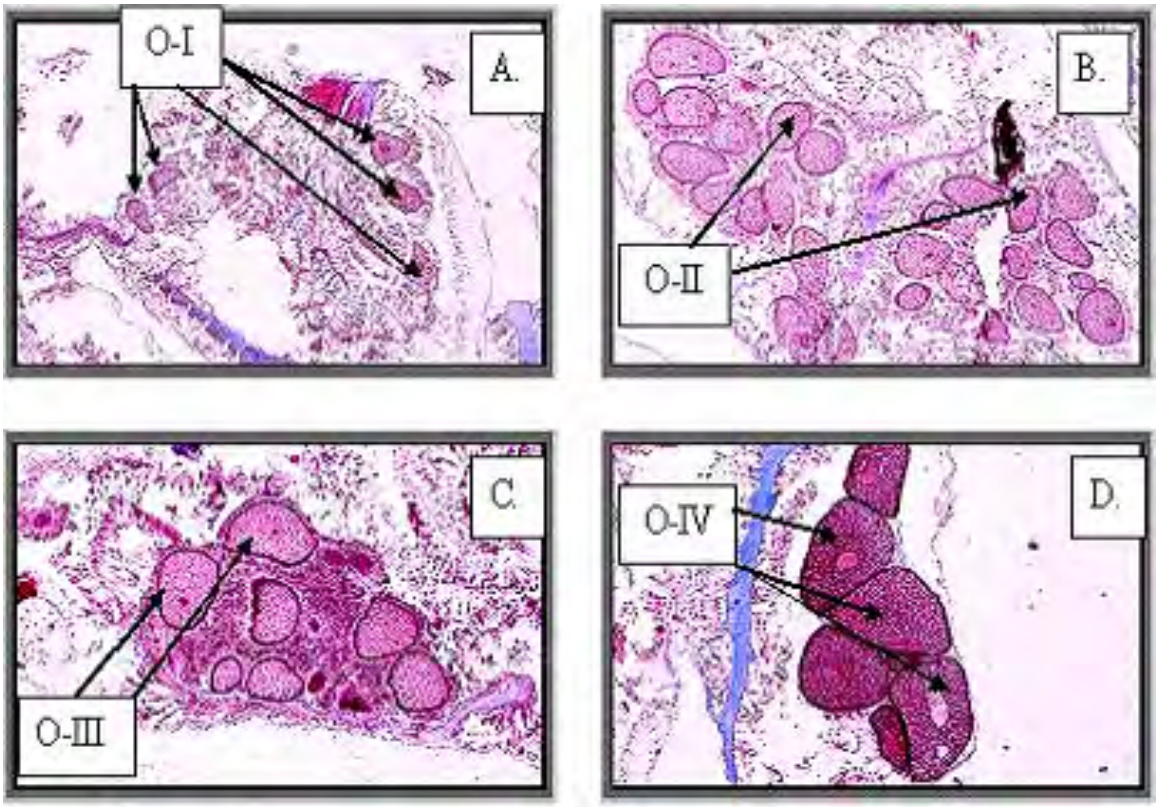


Figure 15. Different stages of oocyte (O) development (O-I to O-IV), showing patterns of spatial arrangement of oocytes and spermatocytes in *M. aliciae*. A and C show an hermaphrodite mesentery and B and D show female mesentery.

Stage II were 15.38 (± 3.13) µm in diameter. Stage III oocytes were observed, and were observed for about 7 months. In March, 80% of sampled colonies were in stage III, with an average diameter of 24.6 (± 3.23) µm. Stage III oocytes are significantly larger than stage II oocytes ($p < 0.0001$) (Table 12; Figure 16). In February, oocytes reached their largest size with an average diameter of 31.93 (± 3.74) µm ($p < 0.0001$) (Table 12; Figure 16). Between February, and March, 60% of the sampled colonies were at this stage. Samples collected in November 2004 also showed the presence of oocytes at different developmental stages within the mesenteries (Figure 20), corroborating the mixed arrangement of oocytes within mesenteries.

Table 12. Non parametric one-way ANOVA test (Kruskal Wallis) for oocyte diameters in *M. aliciae*. Different capital letters indicate significant differences ($p < 0.0001$).

Variable	Stage	N	Media	D.E.	Median	Average range	
Diameter	I	137	5.03	2.29	4.71	69.71	gl= 3
Diameter	II	159	14.52	2.64	14.45	217.92	C= 1.00
Diameter	III	151	23.69	3.33	23.52	376.92	H= 541.52
Diameter	IV	146	31.93	3.74	31.65	513.75	p< 0.0001

Trat.	Ranks				
Stage I	69.71	A			
Stage II	217.92		B		
Stage III	376.92			C	
Stage IV	513.75				D

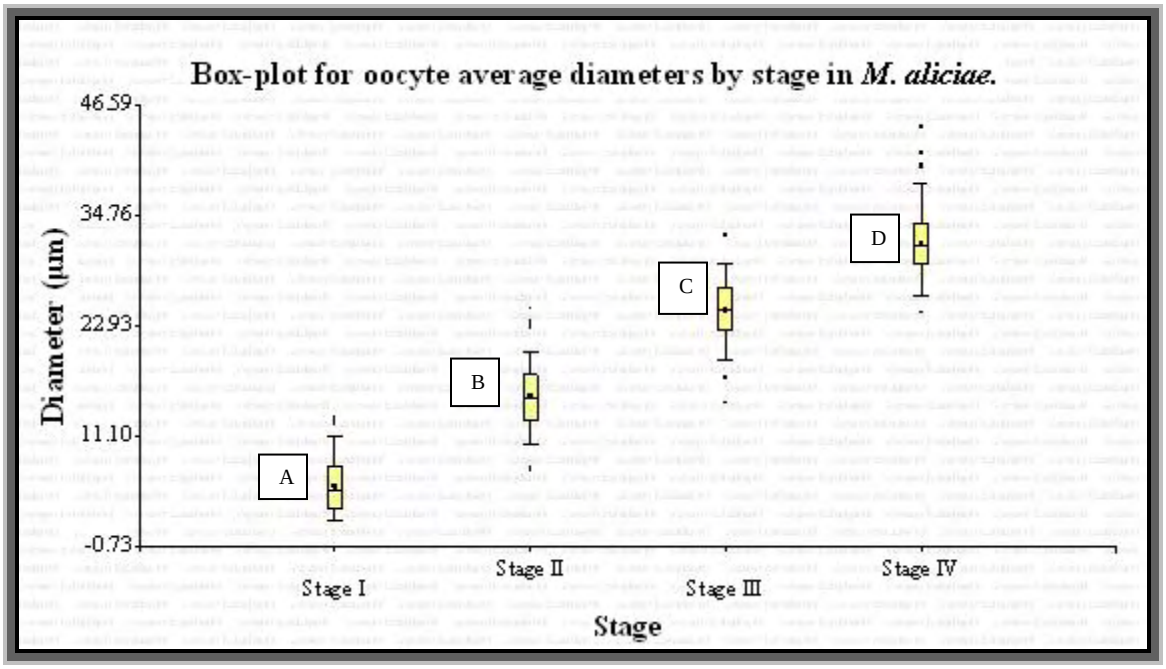


Figure 16. Box-plot for oocyte average diameters by stage in *M. aliciae*. Y axis diameter measurements in (µm), X axis developmental stages I-IV. Different capital letters (A-D) indicate significant difference among the stages.

B.5. *M. aliciae* spermatogenesis

This species had a single cycle of spermatogenesis, with different stages of oocytes present within the mesenteries at all times (Figure 17). Spermatogenesis began in June, with 40% of colonies in stage I (Figure 17-A and Figure 19). Stage II was observed a month after stage I (Figure 19). By October and November 70% and 80% of the colonies were at stage II, respectively (Figure 19). Stage III oocytes were first observed in August, a month after the onset of stage II. Spermatocytes remained in phase II for 5 months; by December, 80% of the colonies had spermatocytes in stage III a few in stage IV oocytes were observed (Figure 19). In March 70% of the colonies displayed spermatocytes in stage IV.

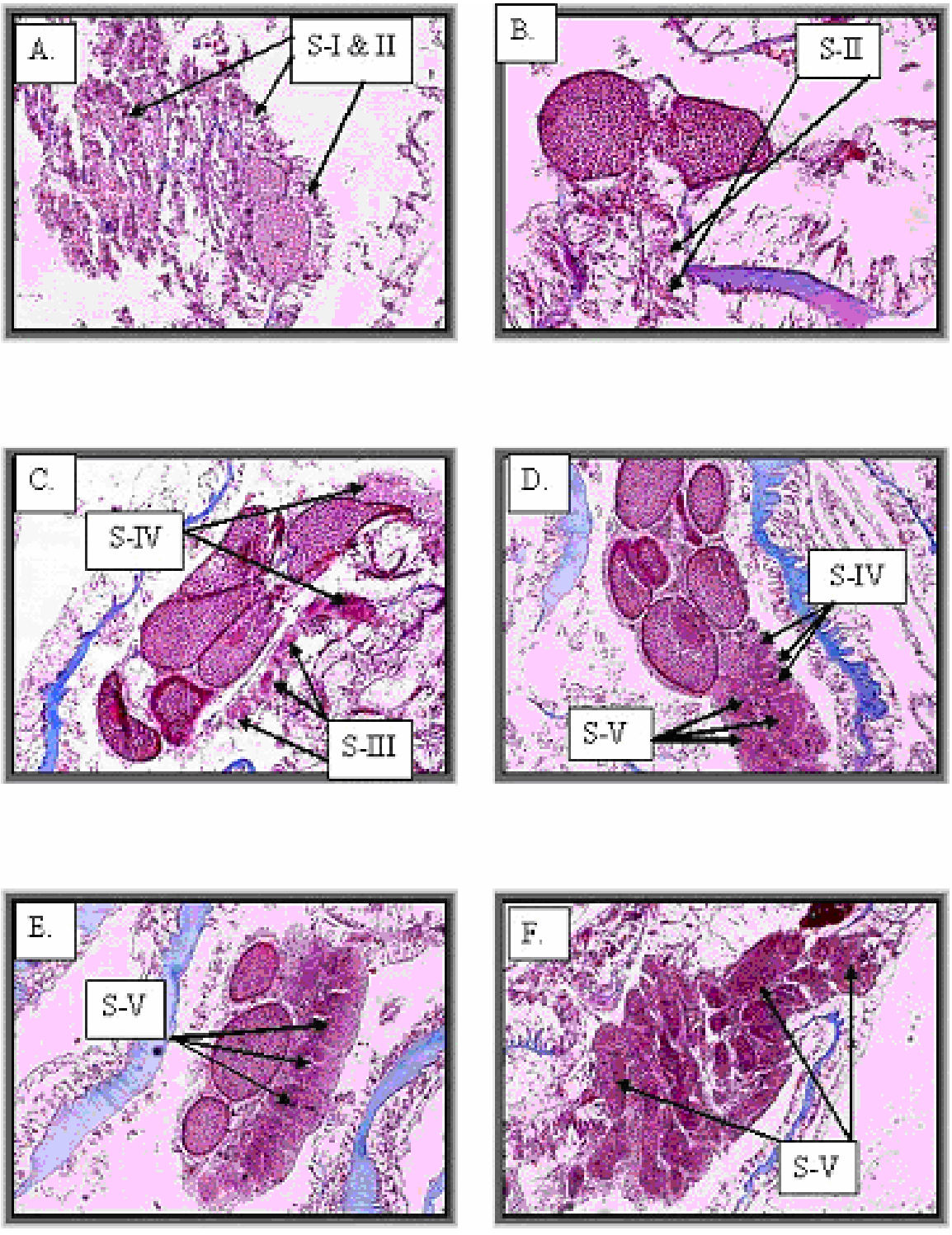


Figure 17. Spermatocyte (S) developmental stages (S-I to S-V), and arrangement within the mesentery in *M. aliciae* (magnification 4x). Oocytes surrounded or intermingled with spermatocytes A and C; Oocyte at the bottom of mesenteries with spermatocytes located towards the coelenteron B and D; male mesentery F.

B.6. *M. aliciae* embryogenesis

Tissue samples for *Mycetophyllia aliciae* did not show embryos in stage I, only eleven stage II larvae were observed (average diameter of 32.77 (± 4.15) µm), between March and April (Figure 18 and Figure 19). *M. aliciae* was the only species in which larvae of stages III and IV with invaginations and mature septas were not observed (Figure 18 and Figure 19).

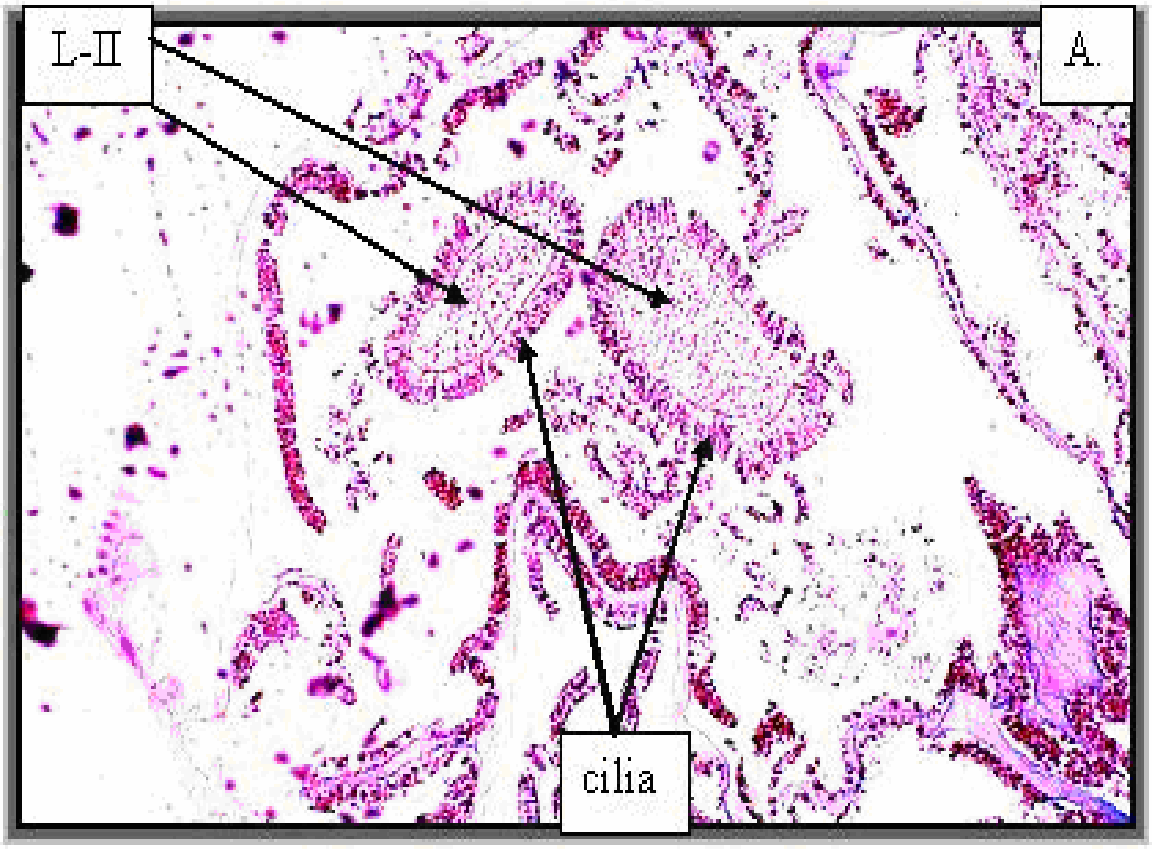


Figure 18. Larvae (L) developmental stage (L-II) within mesenteries in *M. aliciae* (magnification in 4x). The species only shows larvae in stage II, with similar characteristics as oocytes but free from mesenteries.

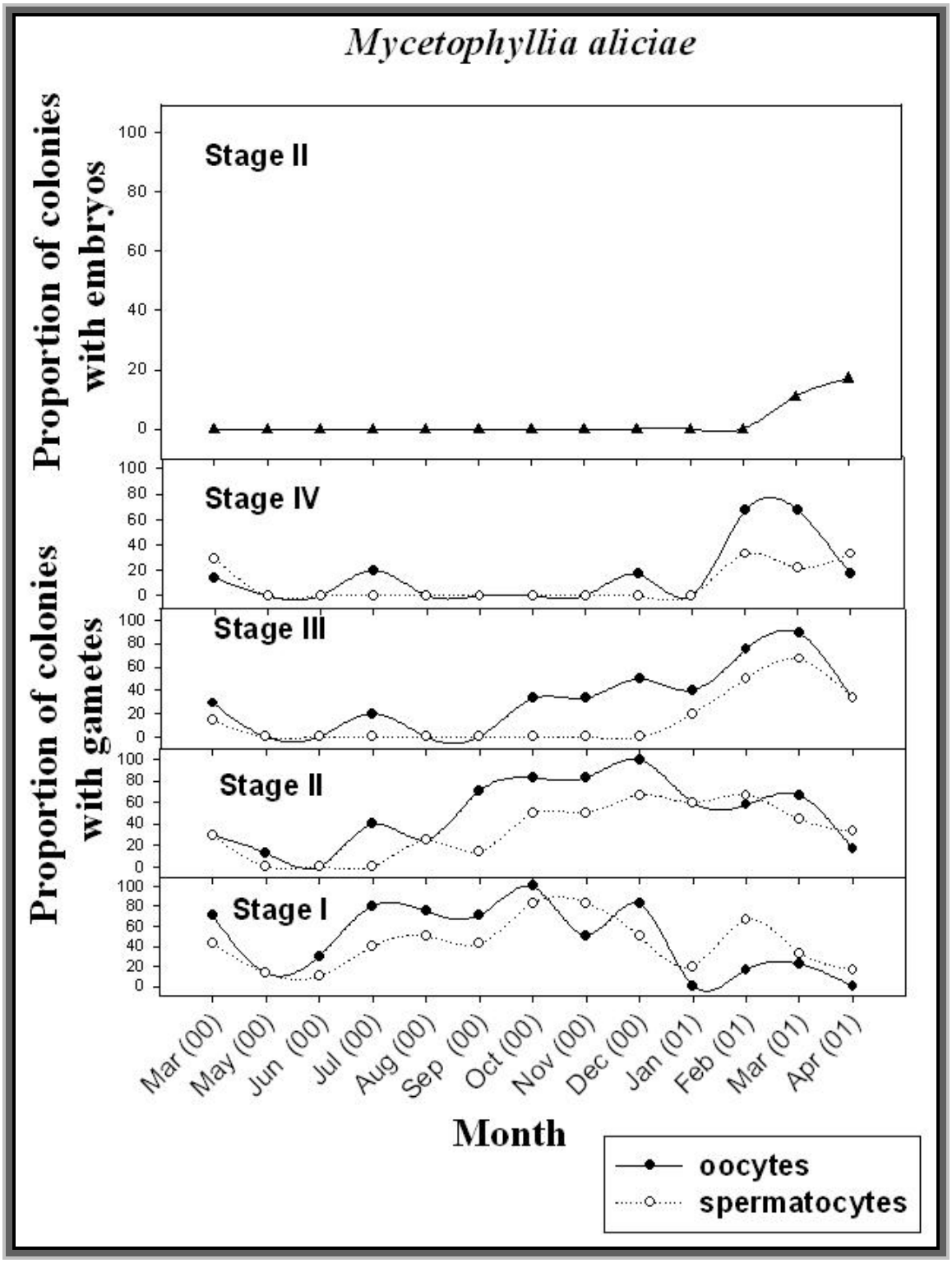


Figure 19. Representative reproductive cycle for *M. aliciae*: Period shown is from March 2000 to April 2001, when monthly samples were taken. Stages I-IV are categories of increasing gametes and embryo maturity, with IV being ripe. Y-axis is the percent of colonies with gametes and embryos in each stage of development.

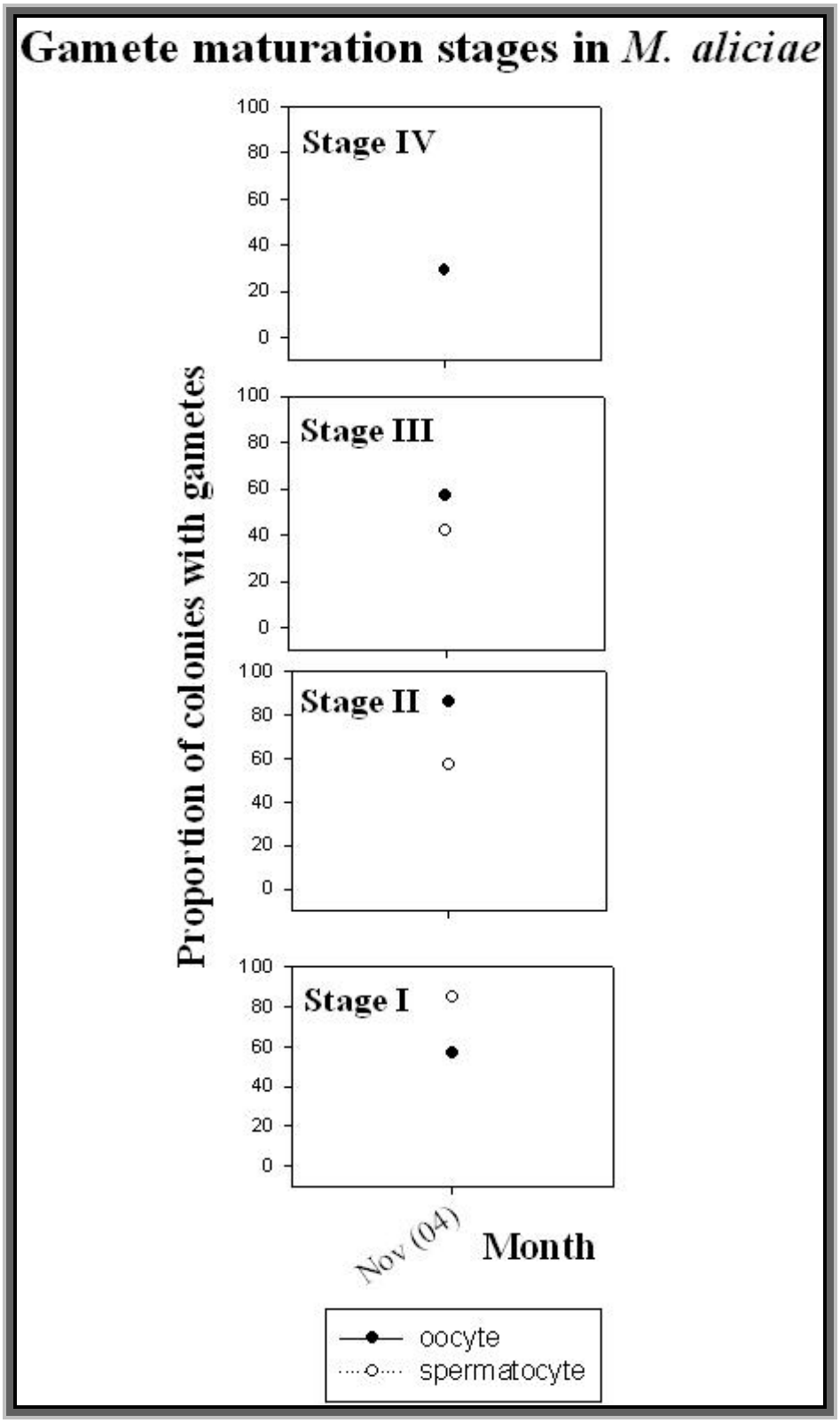


Figure 20. Gamete maturation stages for *M. aliciae.* Period shown is for November 2004. Stages I-IV are categories of increasing gamete maturity, with IV being ripe. Y-axis is the percent of colonies with gametes in each stage of development.

B.7. *M. lamarckiana* oogenesis

Like *Mycetophyllia ferox* and *M. aliciae, M. lamarckiana* had one cycle of oogenesis per year, with different stage oocytes present within the mesenteries at all times. The onset of oogenesis was in August, and remained active for 11 months (Figure 21 and Figure 26). Stages I and II developed simultaneously, lasting about 10 months (Figure 26).

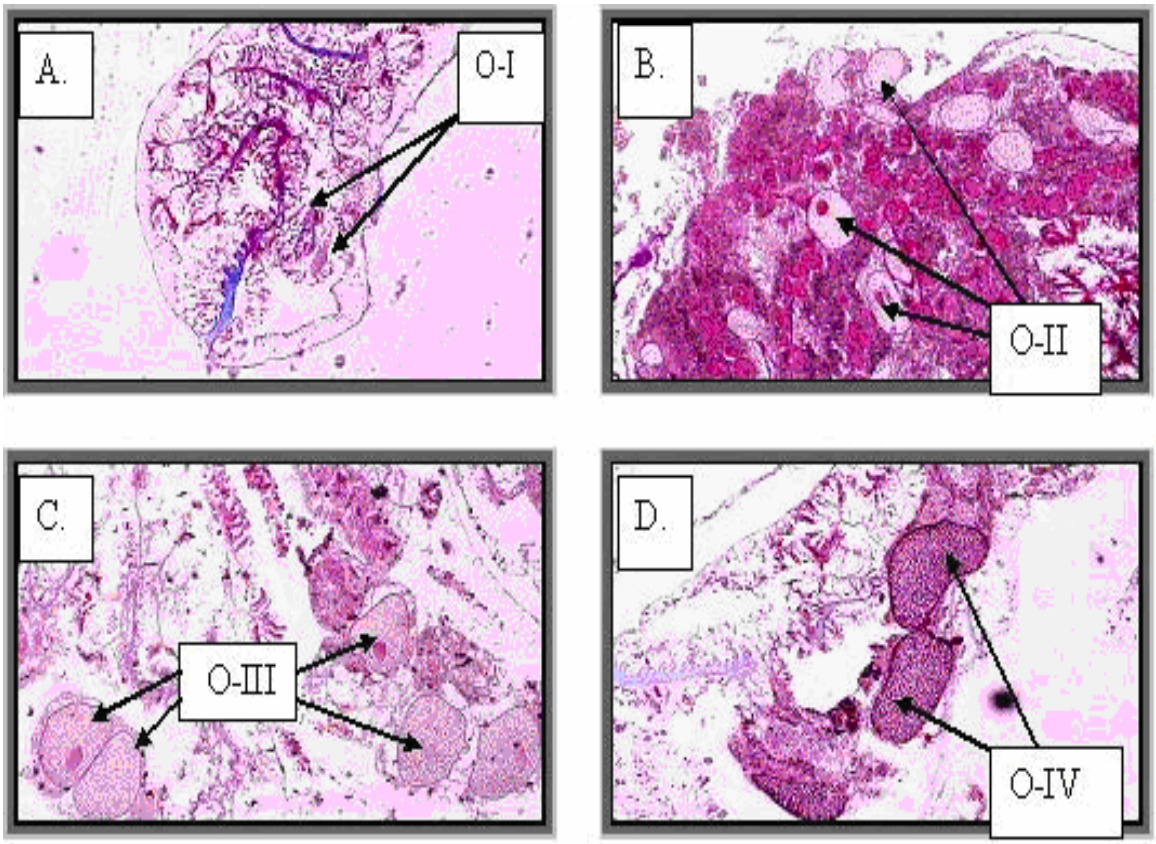


Figure 21. Different stages of oocyte (O) development (O-I to O-IV) showing pattern of spatial arrangement of oocytes and spermatocytes in *M. lamarckiana.* All mesenteries are hermaphrodites, C and D spermatocytes parallel to oocytes.

By September, 100% of sampled colonies were in stage I (Table 13; Figure 22), and by November a 100% of the colonies were in stage II (Figure 26). Oocytes in stage I were 8.2 (± 1.94) µm in average diameter significantly smallest than oocyte in stage IV (Kruskal Wallis, $p < 0.0001$). Oocytes in stage II were 14.78 (± 3.22) µm (Figure 21-B). Oocytes in stage III were observed from December to April, when they were very scarce. In January, 80% of sampled colonies had oocytes in stage III (Figure 26) with an average diameter of 25.67 (± 3.00) µm. By January, oocytes were at their largest size (Kruskal Wallis, $p < 0.0001$), with average diameter of 32.39 (± 2.79) µm. Oocytes in stage IV were observed for four months, from January to April, with 60% of the colonies showing the stage.

Table 13. Non parametric one-way ANOVA test (Kruskal Wallis) for oocyte diameters in *M. lamarckiana*. Different capital letters indicate significant differences ($p < 0.0001$).

Variable	Stage	N	average	SD	Median	Average range	
Diameter	I	149	8.21	1.94	8.42	80.07	gl= 3
Diameter	II	208	14.78	3.22	14.55	250.56	C= 1.00
Diameter	III	114	25.67	3.00	25.63	415.67	H= 454.40
Diameter	IV	51	32.39	2.79	32.71	491.55	P< 0.0001

Trat.	Ranks				
Stage I	80.07	A			
Stage II	250.56		B		
Stage III	415.67			C	
Stage IV	491.55				D

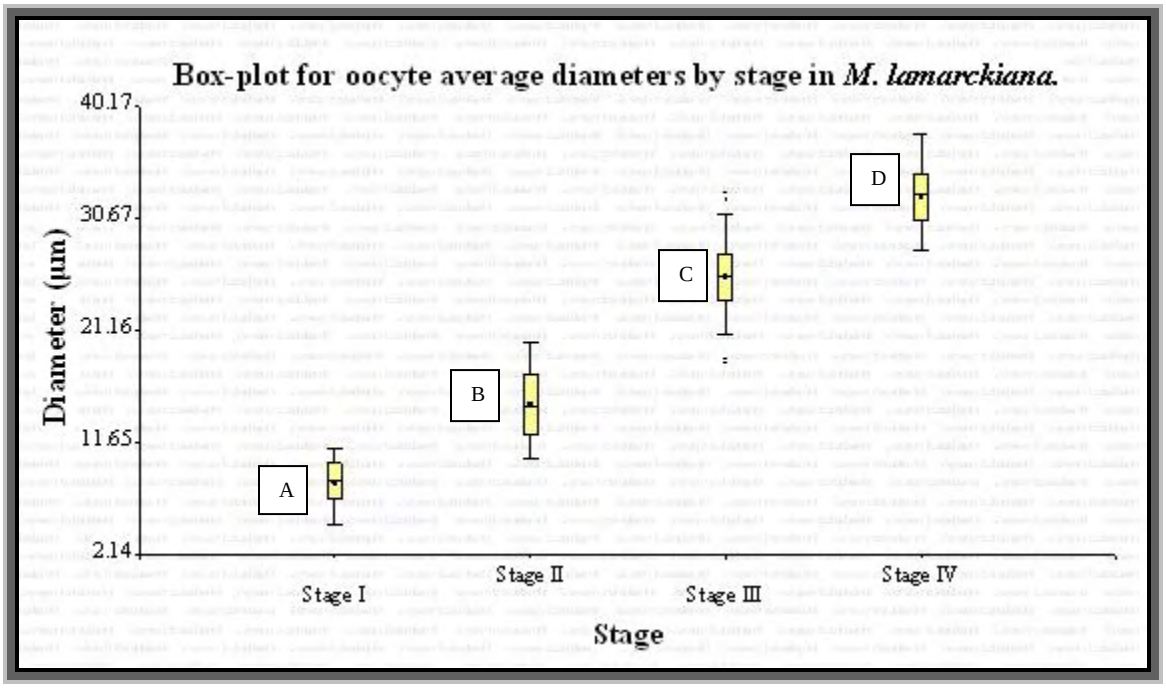


Figure 22. Box-plot for oocyte average diameters by stage in *M. lamarckiana*. Y axis diameter measurements in (µm), X axis developmental stages I-IV. Different capital letters (A-D) indicate significant difference among the stages.

B.8. *M. lamarckiana* spermatogenesis

The spermatogenesis in *M. lamarckiana* began simultaneously with oogenesis, by the month of August (Figure 23 and Figure 26). The changes of stage I through V spermatocytes were in synchrony, with the changes of oocyte stages. Stages I and II spermatocytes had a duration of 8 and 10 months respectively (Figure 26). By November, 100% of sampled colonies were in stage I, and by December, 80% of sampled colonies were in stage II (Figure 26). After stage III, changes to stage IV and V were faster, not lasting more than a month in each developmental stage (Figure 26). Spermatocytes reached maturation simultaneously with oocytes for the same breeding season in January.

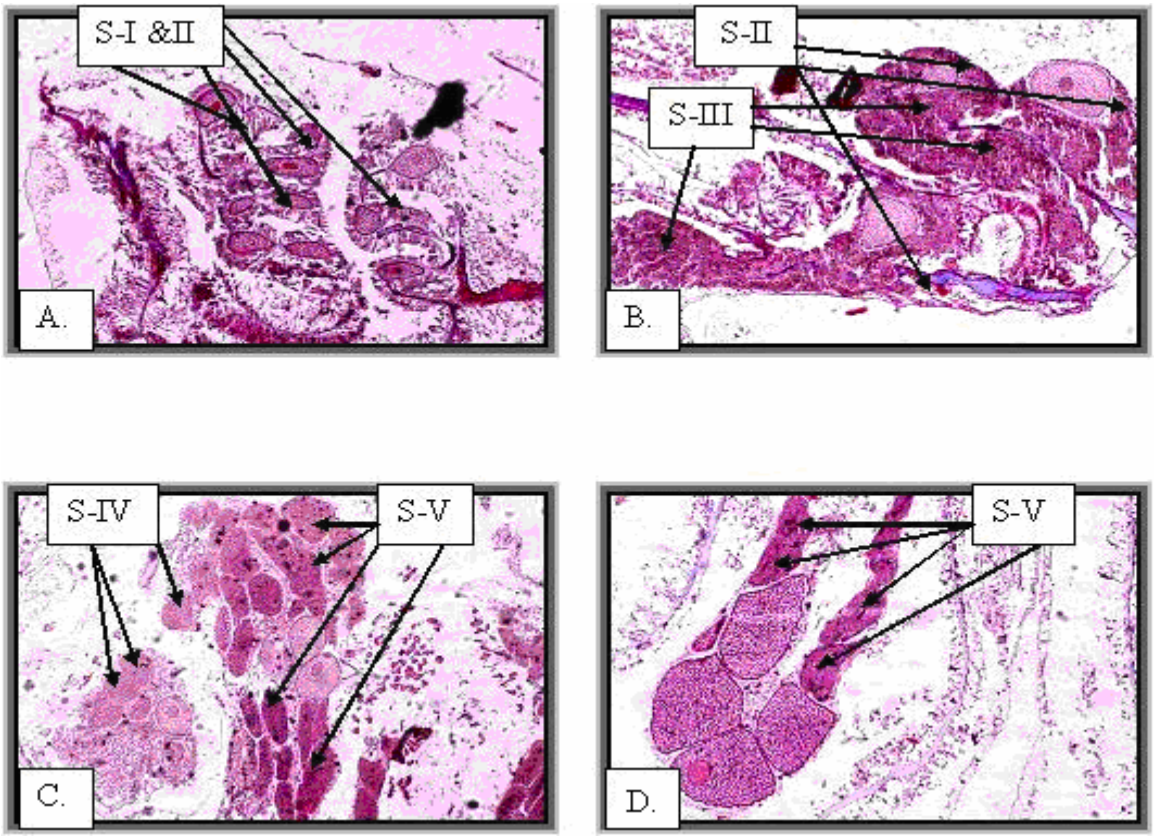


Figure 23. Spermatocyte (S) developmental stages (S-I to S-V) and arrangement within the mesentery in *M. lamarckiana* (magnification 4x). A and B oocyte surrounded or intermingled with spermatocytes, C spermatocytes surrounding oocytes, D oocyte at the bottom of mesenteries with spermatocytes located towards the coelenteron.

B.9. *M. lamarckiana* embryogenesis

Longitudinal sections of tissue samples (Figure 24 and Figure 26) showed that this species developed larvae from August to April, showing one extended period of planula development per year (Figure 26). Embryogenesis was observed when embryos were in gastrula stage (stage I), with invagination of ectodermis within the mesenteries from August to January (Figure 24-A and Figure 26). By February, 45% of colonies had planulae in stage II, with similar size to the oocytes with an average diameter of 32.94 (± 6.23) µm (Figure 24 and Figure 26). Stage I was significantly smaller than stages II, III and IV ($p < 0.0001$) (Table 14; Figure 25). By March, 40% of larvae were in stage II, with an average

diameter of 45.20 (± 6.12) µm (Figure 26). These larvae had mesoglea, cilia and did not stain red (Figure 24-B-C). The onset of stage III was in March, showing invaginations of gastrodermis to form mesenteries, and with average diameter of 63.98 (± 10.66) µm (Figure 24-D and Figure 26). In May, 43% of sampled colonies had reached stage IV, with well developed septa and an average diameter of 71.65 (± 17.53) µm (Figure 24-E and Figure 26). Significant difference in size among larvae of stage II, III and IV was not seeing, but there was significant difference among these stages and stage I (Kruskal Wallis, $p < 0.0001$) (Table 14; Figure 25). This species developed 1 to 3 larvae per mesentery, within the same mesentery or in different mesenteries with gametes.

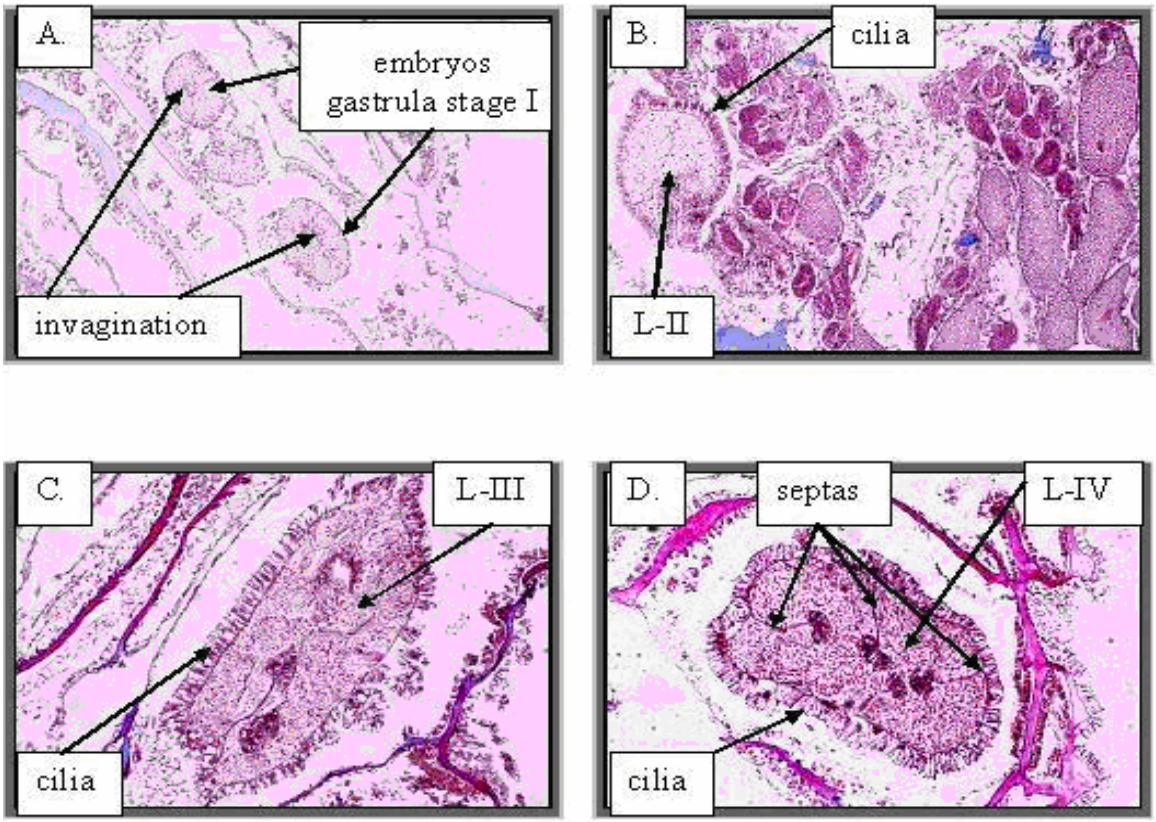


Figure 24. Larvae (L) developmental stages (L-I to L-IV) within mesenteries in *M. lamarckiana* (magnification in 4x). A stage I gastrula with invagination of ectodermis and similar size as oocytes but free from mesenteries, B stage II larvae with mesoglea surrounded by cilia, D stage III, bigger with invaginations of mesoglea and endoderm and E larvae with well developed septa.

Table 14. Non parametric one-way ANOVA test (Kruskal Wallis) for larvae diameters in *M. lamarckiana*. Different capital letters indicate significant differences ($p < 0.0001$).

Variable	Stage	N	Average	SD	Median	Average range	
Diameter	I	26	32.94	6.23	34.62	13.88	gl= 3
Diameter	II	5	45.20	6.12	48.30	28.40	C= 1.00
Diameter	III	8	63.98	10.66	64.57	36.50	H= 30.50
Diameter	IV	5	71.65	17.53	75.72	39.00	p < 0.0001

Trat.	Ranks			
Stage I	13.88	A		
Stage II	28.40		B	
Stage III	36.50		B	
Stage IV	39.00		B	

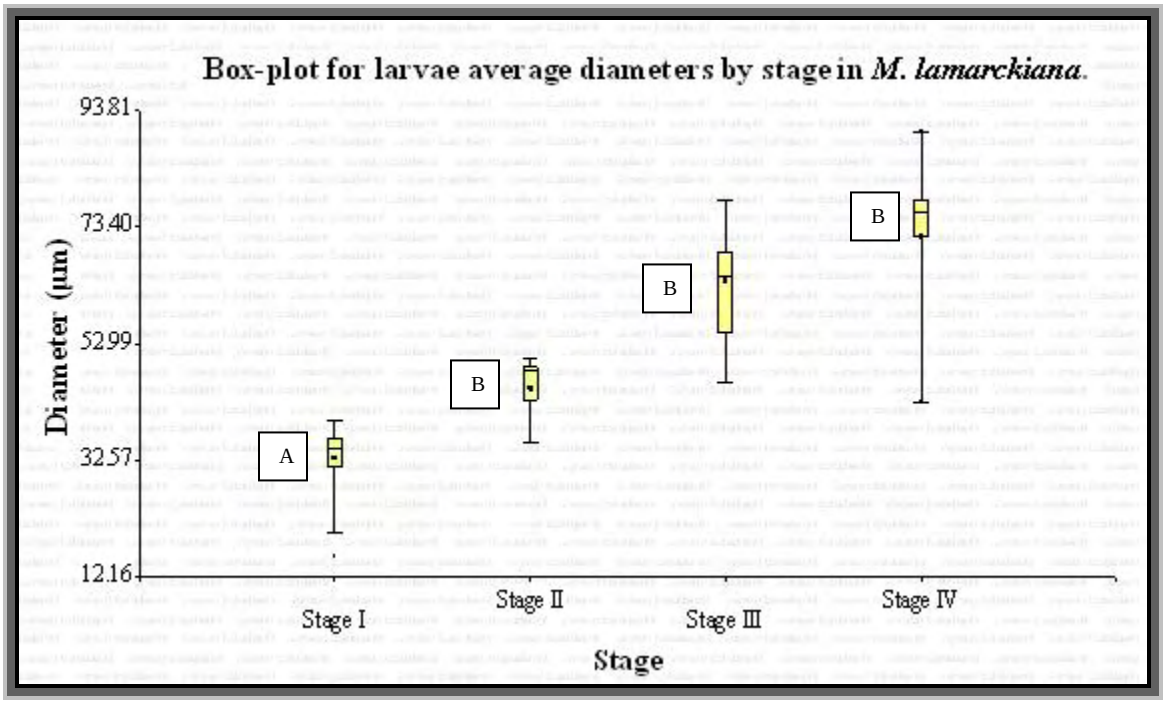


Figure 25. Box-plot for average larvae diameters by stage in *M. lamarckiana*. Y axis diameters measurements in (µm), X axis developmental stages I-IV. Different capital letters (A-C) indicate significant difference among the stages.

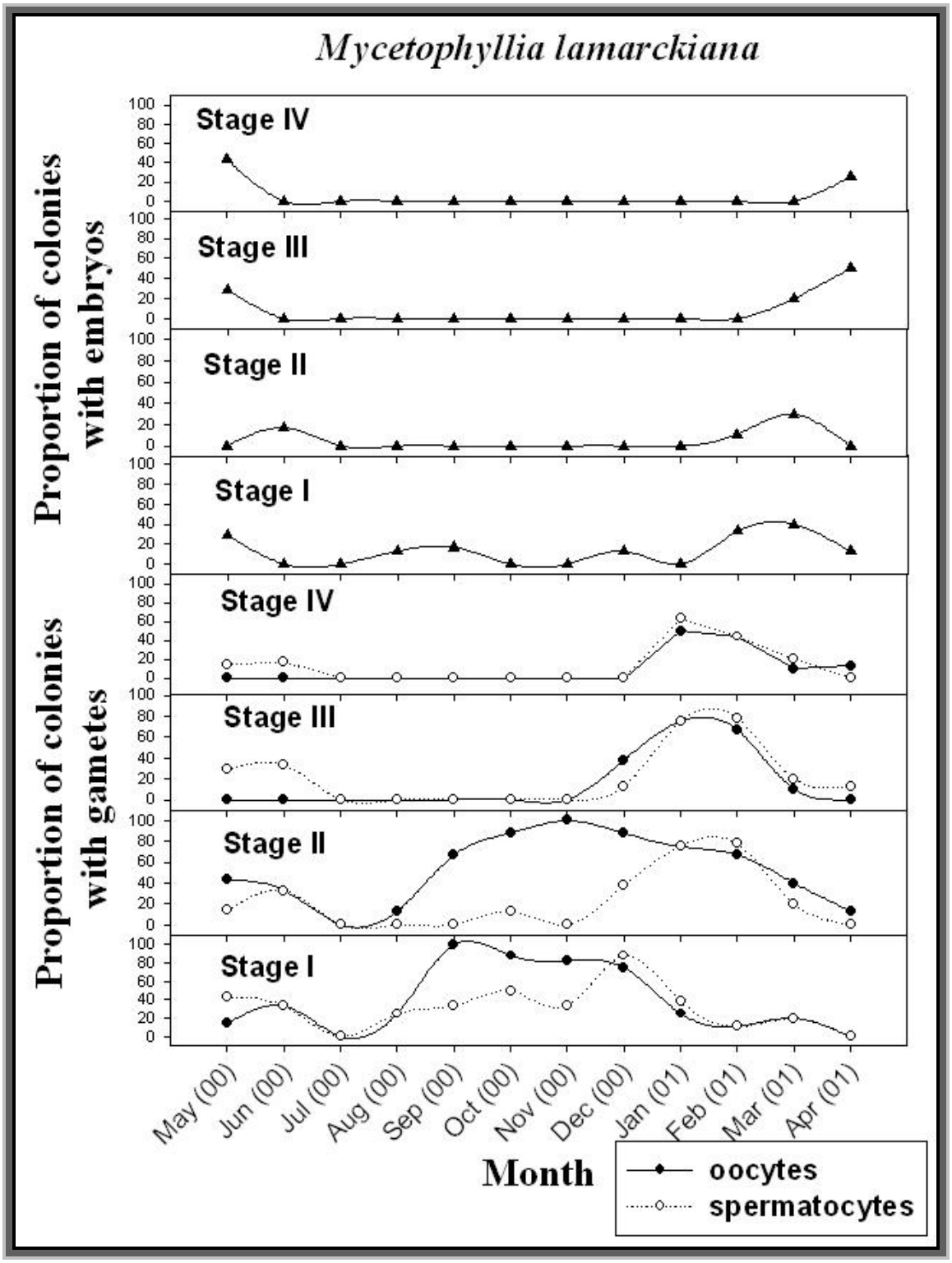


Figure 26. Representative reproductive cycle for *M. lamarckiana*: Period shown is from May 2000 to April 2001, when monthly samples were taken. Stages I-IV are categories of increasing gamete and embryo maturity, with IV being ripe. Y-axis is the percent of colonies with gametes and embryos in each stage of development.

B.10. *M. danaana* oogenesis

Histological samples showed a single cycle of oogenesis per year, which lasted throughout the year, with the onset of oogenesis in March (Figure 27 and Figure 32). This species has different stage oocytes within the mesenteries at all times. Oocytes in stages I and II were observed throughout the year (Figure 32). One hundred percent of colonies sampled in August, October and January showed oocytes in stage I, and stage II oocytes were observed from September to January (Figure 27 and Figure 32). Stage I oocytes had an average diameter of 6.55 (± 1.76) µm; stage II oocytes were 14.37 (± 3.11) µm. By

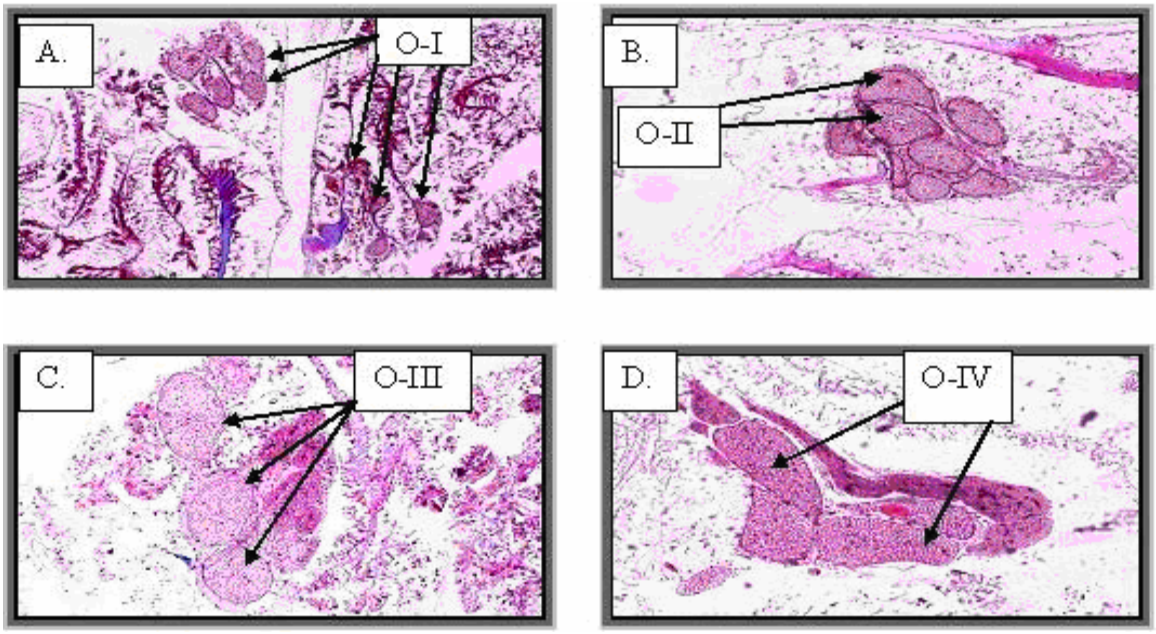


Figure 27. Different stages of oocyte (O) development (O-I to O-IV) showing pattern of spatial arrangement of oocytes and spermatocytes in *M. danaana*. In A, B, C and D all mesenteries are hermaphrodites and show oocytes surrounded or intermingled with spermatocytes.

December, oocytes were nearly at stage III, which were subsequently observed for 4 months. Stage III oocytes were 22.56 (± 3.48) µm in average diameter (Figure 27-C). In January, and February, 100% of sampled colonies were at stage III changing quickly to

the last stage IV, with significantly larger eggs (Kruskal Wallis, $p < 0.0001$) (Table 15: Figure 28) by February. Oocytes in stage IV were about 28.46 (± 4.84) µm in average diameter (Figure 27-D).

Table 15. Non parametric one-way ANOVA test (Kruskal Wallis) for oocyte diameters in *M. danaana*. Different capital letters indicate significant differences ($p < 0.0001$).

Variable	Stage	N	average	SD	Median	Average range	
Diameter	I	170	6.55	1.76	6.67	87.55	gl= 3
Diameter	II	233	14.37	3.11	14.14	288.41	C= 1.00
Diameter	III	68	22.56	3.49	23.05	429.34	H= 389.06
Diameter	IV	13	28.46	4.84	28.34	468.54	p< 0.0001

Trat.	Ranks			
Stage I	87.55	A		
Stage II	288.41		B	
Stage III	429.34			C
Stage IV	468.54			C

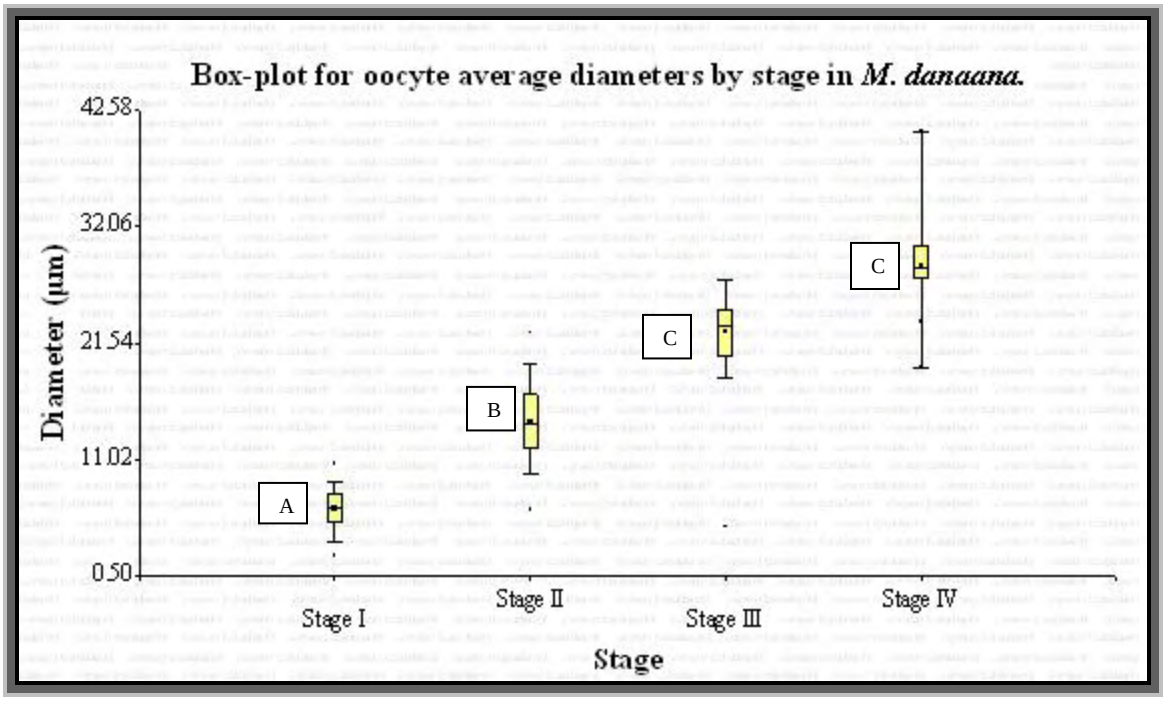


Figure 28. Oocyte average diameters by stage in *M. danaana*. Y axis diameters measurements in (µm), X axis developmental stages I-IV. Different capital letters (A-D) indicate significant difference among the stages.

B.11. *M. danaana* spermatogenesis

Contrary to *M. aliciae*, *M. lamarckiana,* and *M. ferox, M. danaana* seemed to have two cycles of spermatogenesis per year (Figure 29 and Figure 32). The first cycle (May–Feb.) initiated at the same time with oogenesis in May, and the second cycle initiated in February, and ended in June (Figure 32). In both cycles, spermatocytes in stages I and II lasted the same time, 9 months in the first cycle and 4 months in the second cycle (Figure 32 stage I and stage II). By August-November all the colonies had spermatocytes in stages I and II in the first cycle (Figure 32). The changes from stage III to stage V were quickly in January, because spermatocytes require little energy, and resources to be produced. The first cycle of spermatogenesis culminated in February, just before oocytes maturation. When spermatocytes changed to stage III quickly matured to stage V (in December). The second cycle began in February (Figure 32). This cycle was shorter than the first, lasting 4 months, and with changes from one stage to next stage relatively fast, not lasting more than a month. The second cycle culminated just after oocytes maturation in June (Figure 32). Both cycles showed a brief period of overlapping with mature oocytes. The first cycle overlaps during the months of January to March, and the second cycle during the months of March to April (Figure 32).

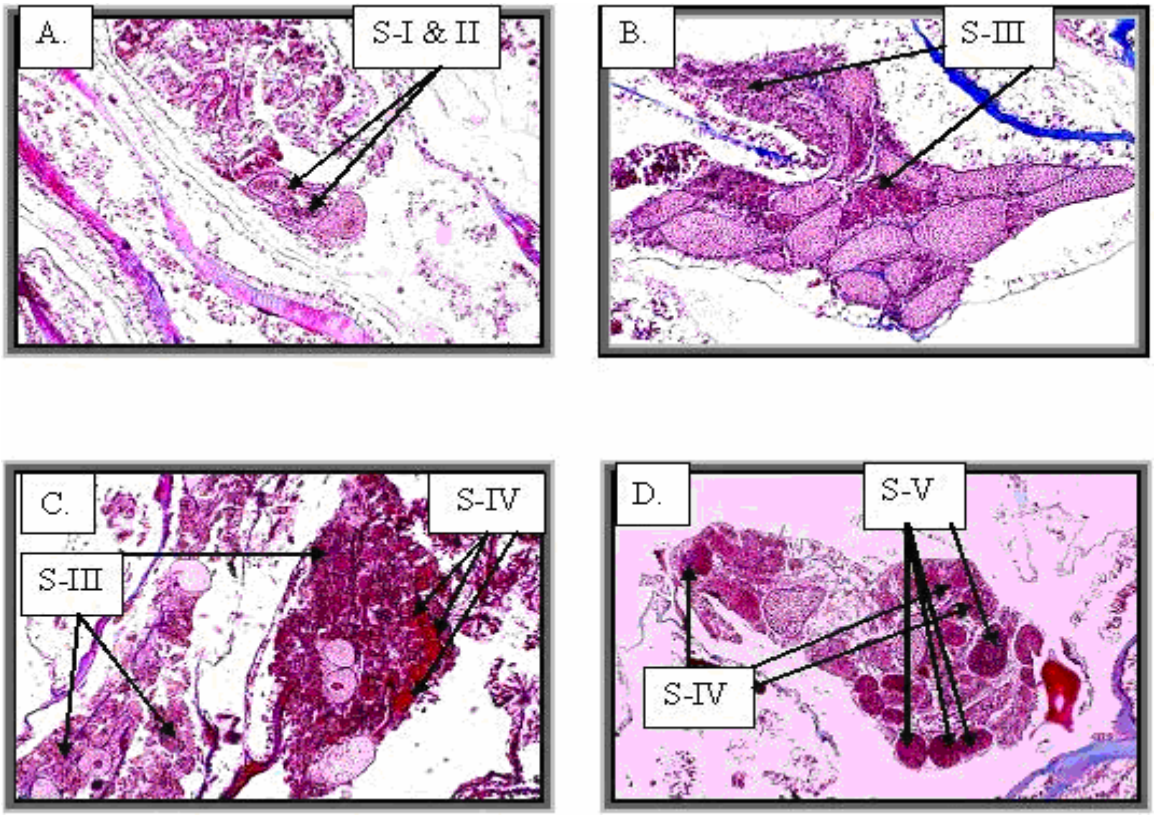


Figure 29. Spermatocyte (S) developmental stages (S-I to S-V) and arrangement within the mesentery in *M. danaana* (magnification 4x). A, B, C and D spermatocyte surrounding oocytes or intermingled with oocytes, F male mesentery.

B.12. *M. danaana* embryogenesis

Longitudinal sections of tissue samples (Figure 30 and Figure 32) showed that this species developed 1 to 3 larvae within a mesentery together or separated from gametes (Figure 30-A). The number of larvae observed at different stages was very small (1 to 3), compared to the number of oocytes produced (1 to 24). As a possible consequence of two spermatogenesis cycles, *M. danaana* displayed planula development through the year, with peak maturation in April. The development was faster from February to May, and was continuous throughout the developmental stages I-IV during winter (Figure 32). From May to February the development was slow, lasting 9 months, with low proportions of planulae in each stage (Figure 32). Stage I larvae had similar to oocytes in size and were

significantly smaller ($p < 0.0001$) (Table 16; Figure 31) than larvae of stages II, III and IV, with an average diameter of 26.77 (± 6.38) µm (Figure 30-A and Figure 32). The stage II was observed from June to August, with mesoglea, cilia, and a mean diameter of 59.73 (± 21.46) µm (Figure 30-B and Figure 32). Stage III, was observed from September to November, with mesenteries forming as invaginations of the mesoglea, and with mean diameter of 67.71 (± 18.94) µm (Figure 30-C and Figure 32). Planula development culminated from February to April, with well developed septa (stage IV), and an average diameter of 82.66 (± 27.10) µm (Figure 30-D and Figure 32). Larvae of stages II to IV did not show significant difference in average diameter ($p < 0.0001$) (Table 16; Figure 31).

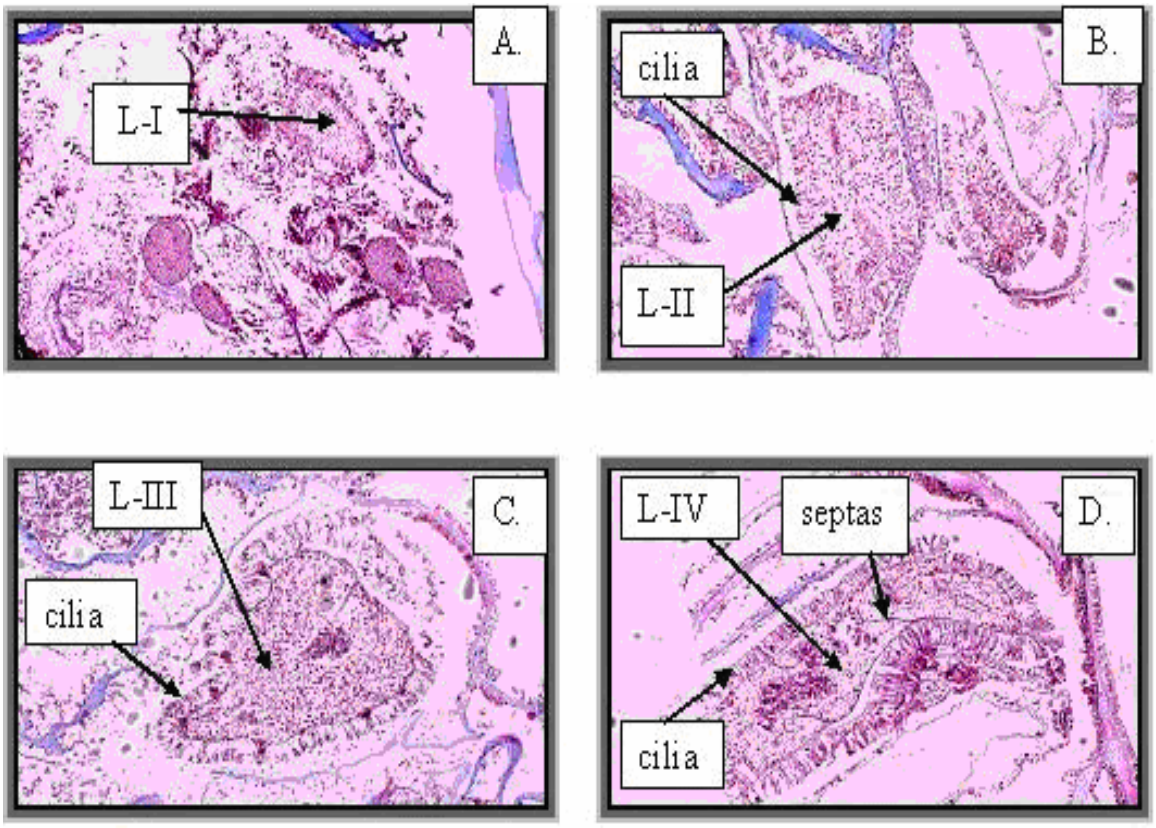


Figure 30. Larvae (L) developmental stages (L-I to L-IV) within mesenteries in *M. danaana* (magnification in 4x). A stage II larvae with mesoglea surrounded by cilia in the same mesentery with oocytes and spermatocytes, B and C stage III larger with invaginations of mesoglea and endoderm and D larvae with well developed septas.

Table 16. Non parametric one-way ANOVA test (Kruskal Wallis) for larvae diameters in *M. danaana*. Different capital letters indicate significant differences ($p < 0.0001$).

Variable	Stage	N	Average	SD	Median	Average range	
Diameter	I	20	26.77	6.38	26.02	11.50	gl= 3
Diameter	II	6	59.74	21.46	60.35	27.67	C= 1.00
Diameter	III	9	67.71	18.94	72.69	29.11	H= 24.17
Diameter	IV	5	82.66	27.10	72.08	32.40	p< 0.0001

Trat.	Ranks			
Stage I	11.50	A		
Stage II	27.67		B	
Stage III	29.11		B	
Stage IV	32.40		B	

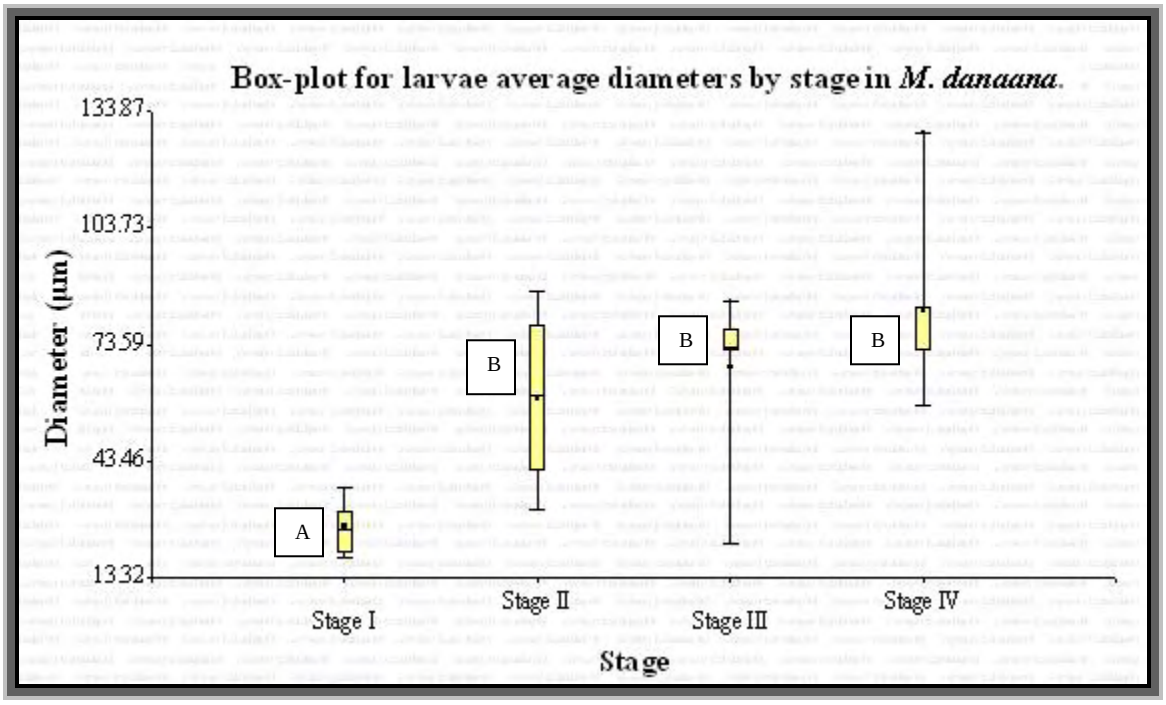


Figure 31. Box-plot for larvae average diameters by stage in *M. danaana*. Y axis diameters measurements in (µm), X axis developmental stages I-IV. Different capital letters (A-C) indicate significant difference among the stages.

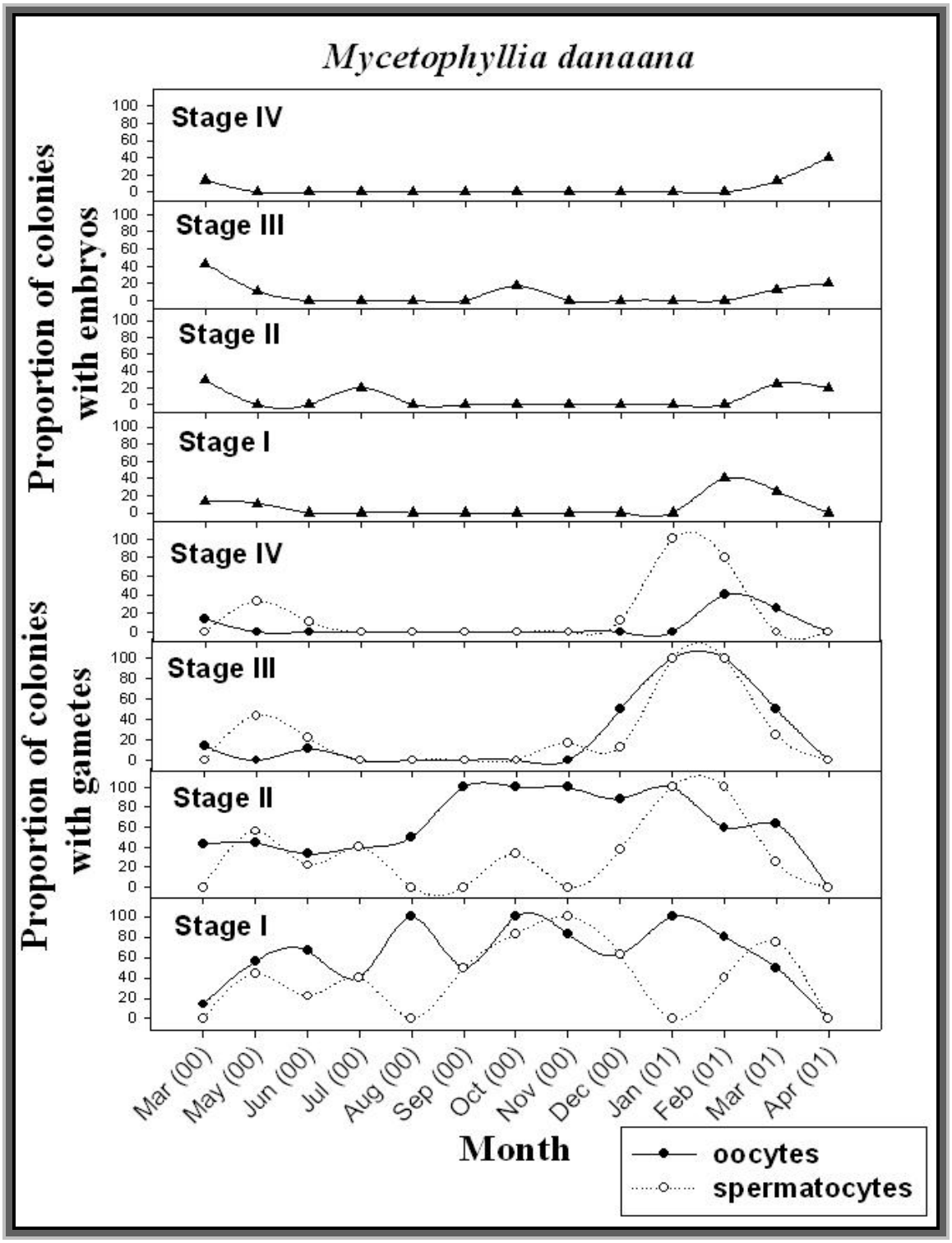


Figure 32. Representative reproductive cycle for *M. danaana*: Period shown is from March 2000 to April 2001, when monthly samples were taken. Stages I-IV are categories of increasing gametes and embryo maturity, with IV being ripe. Y-axis is the percent of colonies with gametes and embryos in each stage of development.

B.13. Statistical comparison of oocyte average diameters by stage among *Mycetophyllia* species.

Significant difference (Kruskal Wallis, $p < 0.0001$) were found in the diameter of oocytes of stage I in *M. aliciae, M. lamarckiana and M. danaana.* The first species produced the smallest oocytes in stage I while *M. lamarckiana* produced the largest oocytes in stage I (Table 17; Figure 33-I). The average oocyte diameter in *M. ferox* was not significantly different compared to *M. danaana* and *M. lamarckiana* in stage I. Oocytes in stage II were significantly different between *M. ferox* and the other 3 species. *Mycetophyllia ferox* produced the smallest oocytes (Kruskal Wallis, $p < 0.0001$) (Table 17; Figure 33-II). No significant difference in stage II oocytes diameter were found among *M. aliciae, M. lamarckiana and M. danaana; M. lamarckiana* produced the largest oocytes in stage III and *M. ferox* the smallest oocytes in stage III. There were no significant differences between *M. aliciae* and *M. danaana* in stage III oocytes diameter (Table 17; Figure 33-III). Oocytes of stage IV showed significant difference (Kruskal Wallis, $p < 0.0001$) among *M. ferox, M. lamarckiana and M. danaana,* with the first producing the smallest oocytes in stage IV and the second producing the largest oocytes. No significant difference (Kruskal Wallis, $p > 0.05$) was found between stage IV oocytes of *M. aliciae* and *M. lamarckiana* (Table 17; Figure 33-IV).

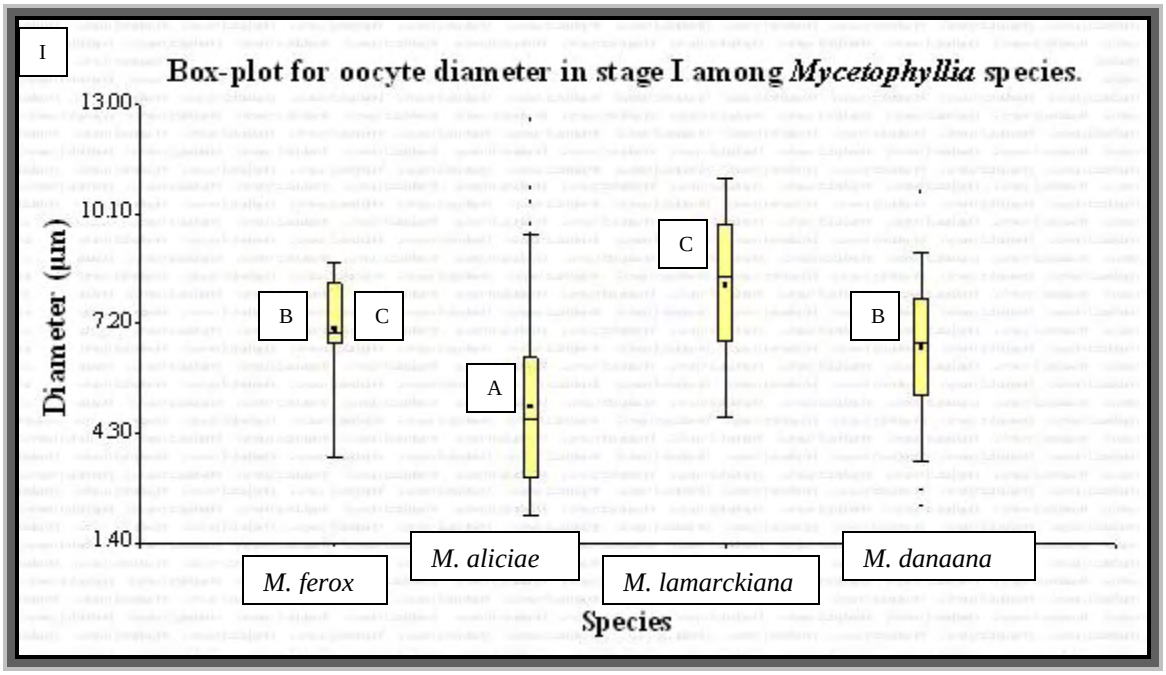
Table 17. Non parametric one-way ANOVA test (Kruskal Wallis) for oocyte diameters by stage among *Mycetophyllia* species. Different capital letters indicate Significant differences ($p < 0.0001$).

Variable	Species		N	Average	SD	Median	average range	p< 0.0001
Diameter	*ali*	I	136	5.00	2.27	4.65	141.01	A
Diameter	*dan*	I	170	6.55	1.76	6.68	229.53	B
Diameter	*fer*	I	15	7.05	1.44	6.97	261.70	B C
Diameter	*lam*	I	149	8.21	1.94	8.42	325.93	C

Variable	Species		N	Average	SD	Median	Average range_	p< 0.0001
Diameter	*ali*	II	159	14.52	2.64	14.45	321.37	B
Diameter	*dan*	II	233	14.37	3.11	14.14	308.00	B
Diameter	*fer*	II	21	10.84	1.29	10.80	93.50	A
Diameter	*lam*	II	208	14.78	3.22	14.55	328.39	B

Variable	Species		N	Average	SD	Median	Average range_	p< 0.0001
Diameter	ali	III	151	23.69	3.33	23.52	170.96	B
Diameter	dan	III	68	22.56	3.49	23.06	144.84	B
Diameter	fer	III	19	14.47	1.68	14.17	1.84	A
Diameter	lam	III	14	25.67	3.00	25.63	230.16	C

Variable	Species		N	Average	SD	Median	Average range_	p< 0.0001
Diameter	*ali*	IV	146	31.93	3.74	31.66	119.00	C
Diameter	*dan*	IV	13	28.46	4.84	28.34	62.31	B
Diameter	*fer*	IV	13	18.83	1.60	18.30	7.38	A
Diameter	*lam*	IV	51	32.39	2.79	32.71	131.30	C



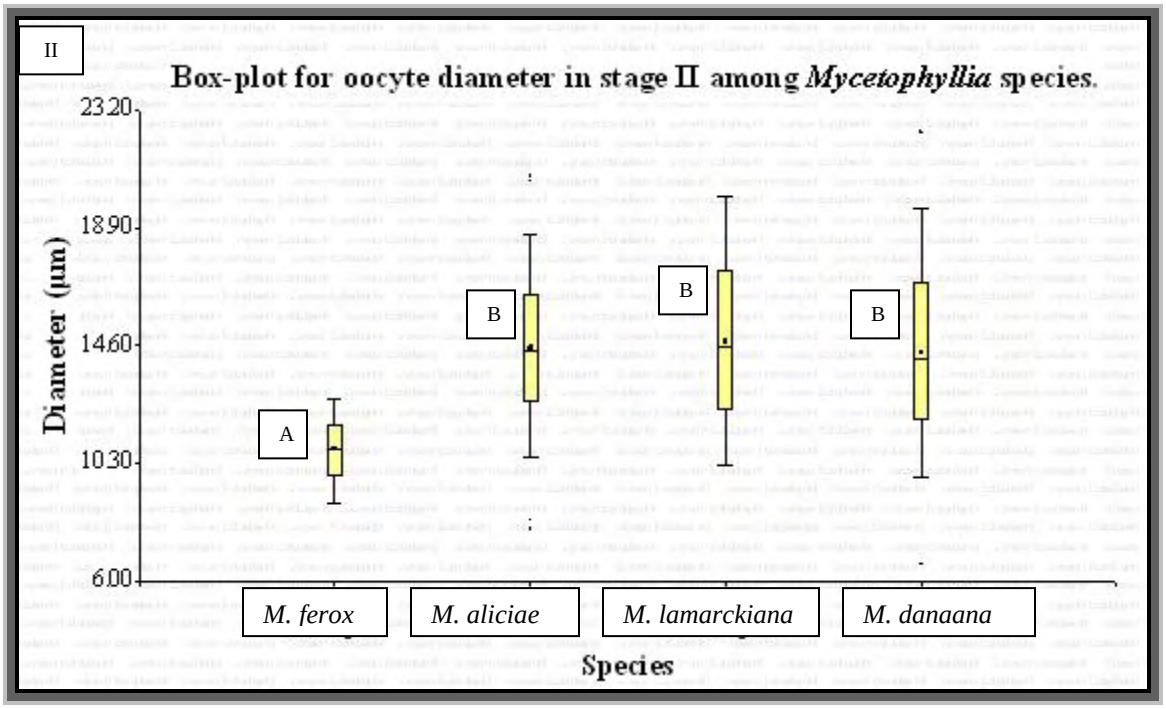
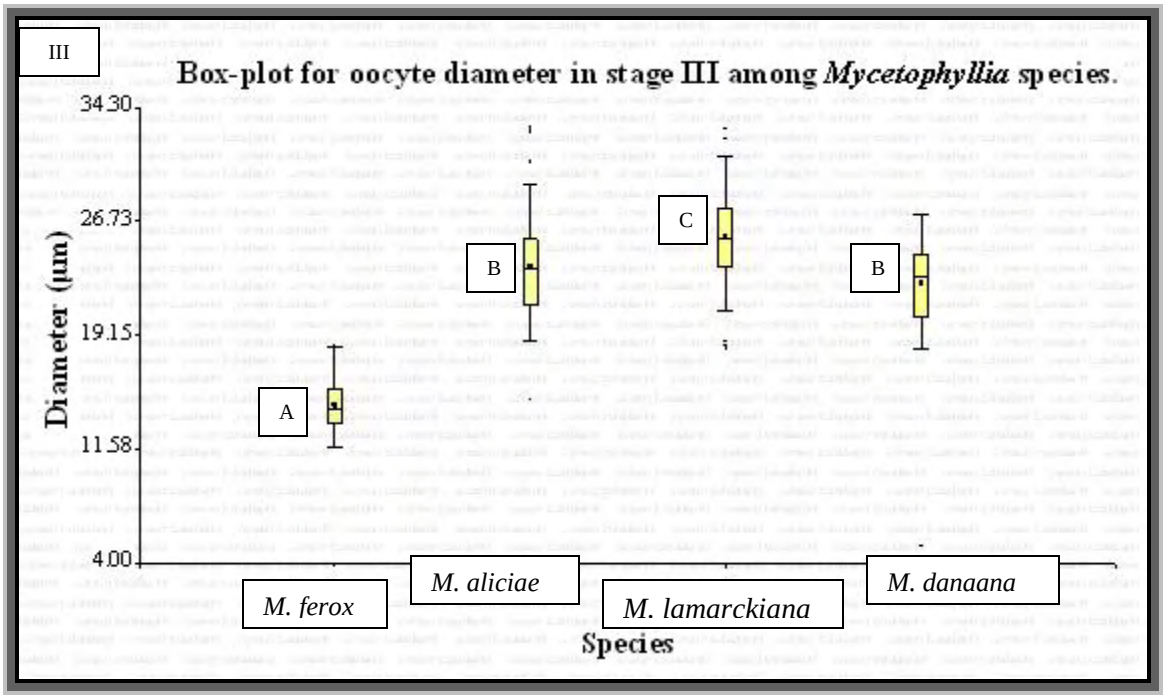


Figure 33. Box-plot comparing oocyte average diameters by stage among *Mycetophyllia* species. Box-plot I show the difference in stage I among the species; Box-plot II shows the differences in stage II among the species. Y axis diameters measurements (µm), X axis developmental stages I-IV. Different capital letters (A-C) indicate significant difference among the stages.



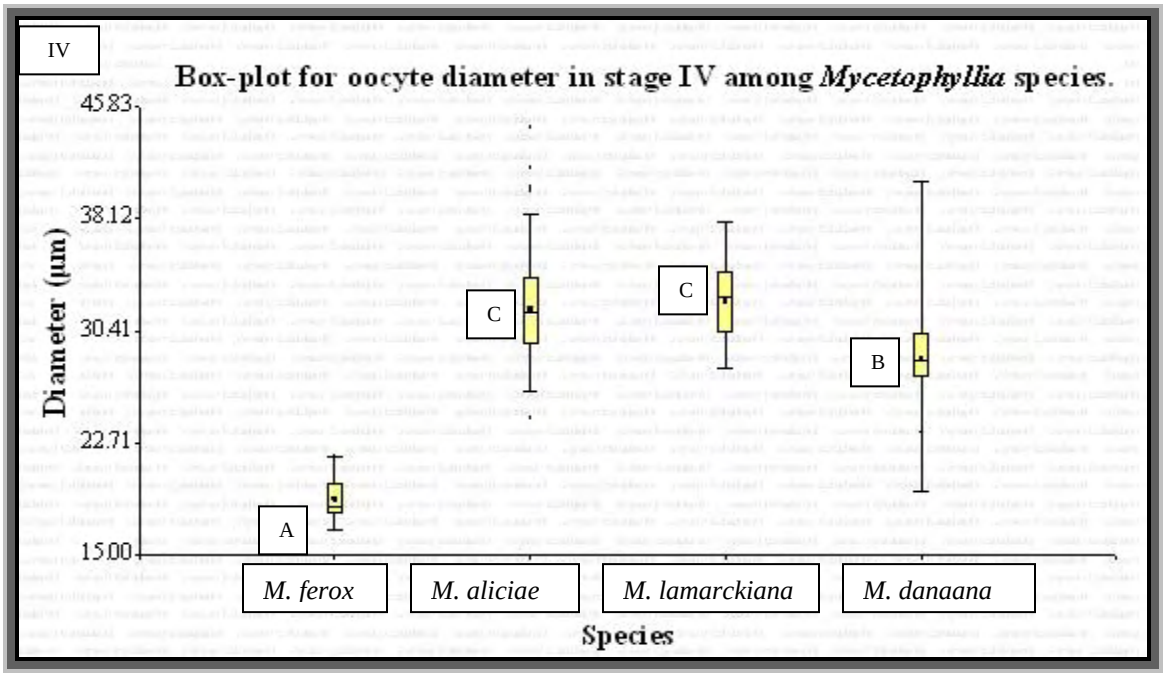


Figure 33. Continuation: Box-plot III shows the differences in stage III among the species; Box-plot IV shows the differences in stage IV among the species. Y axis diameters measurements (µm), X axis developmental stages I-IV. Different capital letters (A-C) indicate significant difference among the stages.

B.14. Statistical comparison of larvae average diameters by stage among *Mycetophyllia* species.

Mycetophyllia ferox and *M. danaana* produced significantly smaller (Kruskal Wallis, $p < 0.0001$) larvae in stage I, while *M. lamarckiana* produced the largest stage I larvae (Table 18; Figure 34-I). There was no significant difference between *M. ferox* and *M. danaana* in stage I. There was significant difference for larvae in stage II ($p< 0.0001$) between *M. ferox*, and *M. danaana*, also between *M. aliciae*, and *M. lamarckiana*. *Mycetophyllia ferox and M. danaana* produced the largest larvae in stage II (Table 18; Figure 34-II). No significant difference in the larvae diameter was found between *M. ferox* and *M. danaana*, or, between *M. aliciae* and *M. lamarckiana*. *M. ferox* produced largest larvae (Kruskal Wallis, $p= 0.0008$) in stage III; *M. lamarckiana* and *M. danaana* produced de smallest larvae (Table 18; Figure 34-III). There was not significant difference between diameters of larvae in stage IV for *M. lamarckiana* and *M. danaana*. Larvae were of similar size for all four species (Kruskal Wallis, $p= 0.7737$) (Table 18; Figure 34-IV).

Table 18. One-way analysis of variance and non parametric one-way ANOVA test (Kruskal Wallis) for larvae diameter by stage among *Mycetophyllia* species. Different capital letters indicate significant differences.

Kruskal Wallis

Variable	Species	N	Media	SD	Median	Average range		p= 0.0033
Diameter	*dan* I	20	26.77	6.38	26.02	18.30	A	
Diameter	*fer* I	3	24.85	1.89	25.26	14.00	A	
Diameter	*lam* I	26	32.94	6.23	34.62	31.42		B

Kruskal Wallis

Variable	Species	N	Media	SD	Median	Average range		p< 0.0001
Diameter	*ali* II	12	32.77	4.15	32.28	8.00	A	
Diameter	*dan* II	6	59.74	21.46	60.35	29.67		B
Diameter	*fer* II	22	57.85	14.68	56.21	29.95		B
Diameter	*lam* II	5	45.20	6.12	48.30	20.40	A	

Variable	N	R^2	R^2 Aj	CV
Diameter	40	0.28	0.24	19.05

Analysis of variance (SC type I)

F.V.	SC	gl	CM	F	p-valor
Model	3010.33	2	1505.16	7.19	0.0023
Species	3010.33	2	1505.16	7.19	0.0023
Error	7747.83	37	209.40		
Total	10758.16	39			

Test: LSD Fisher Alfa:=0.05 DMS:=12.05521

Error: 209.4009 gl: 37

Species	Medias	n		
lam stage III	63.98	8	A	
dan stage III	67.72	9	A	
fer stage III	83.33	23		B

Variable	N	R^2	R^2 Aj	CV
Diameter	18	0.07	0.00	29.37

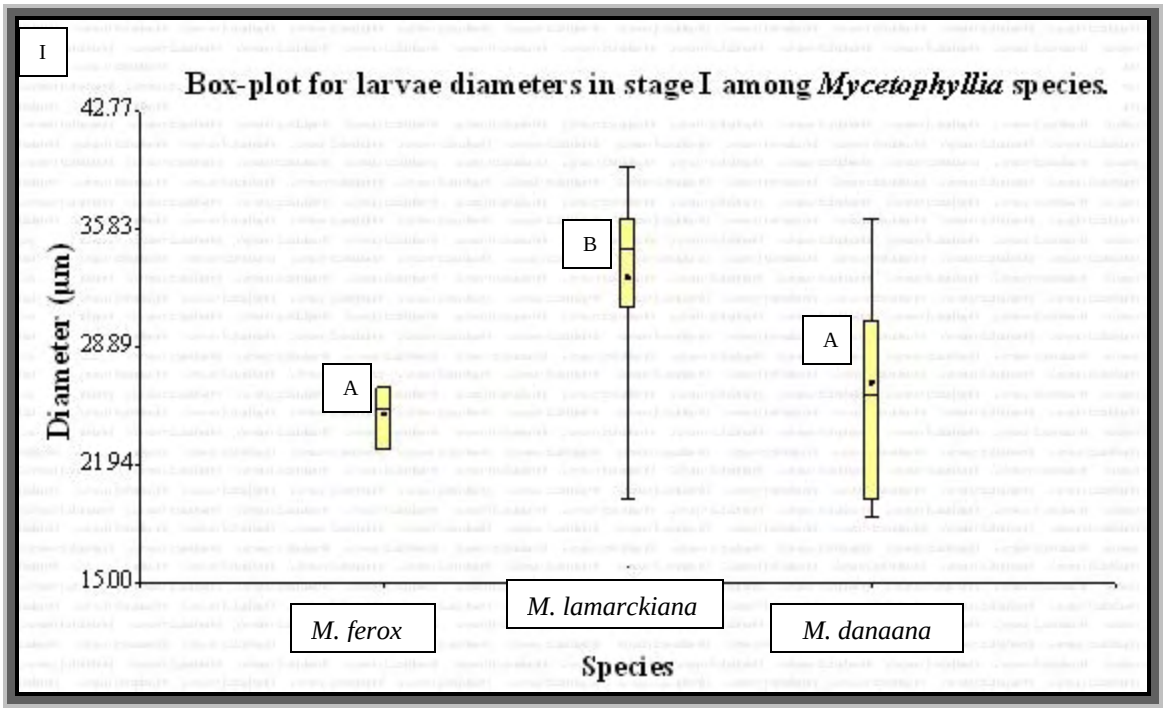
Analysis of variance (SC type I)

F.V.	SC	gl	CM	F	p-valor
Model	589.79	2	294.90	0.52	0.6022
Species	589.79	2	294.90	0.52	0.6022
Error	8429.83	15	561.99		
Total	9019.62	17			

Test: LSD Fisher Alfa: =0.05 DMS: =29.54940

Error: 561.9886 gl: 15

Species	Medias	n		
lam stage IV	71.65	5	A	
dan stage IV	82.66	5	A	
fer stage IV	85.19	8	A	



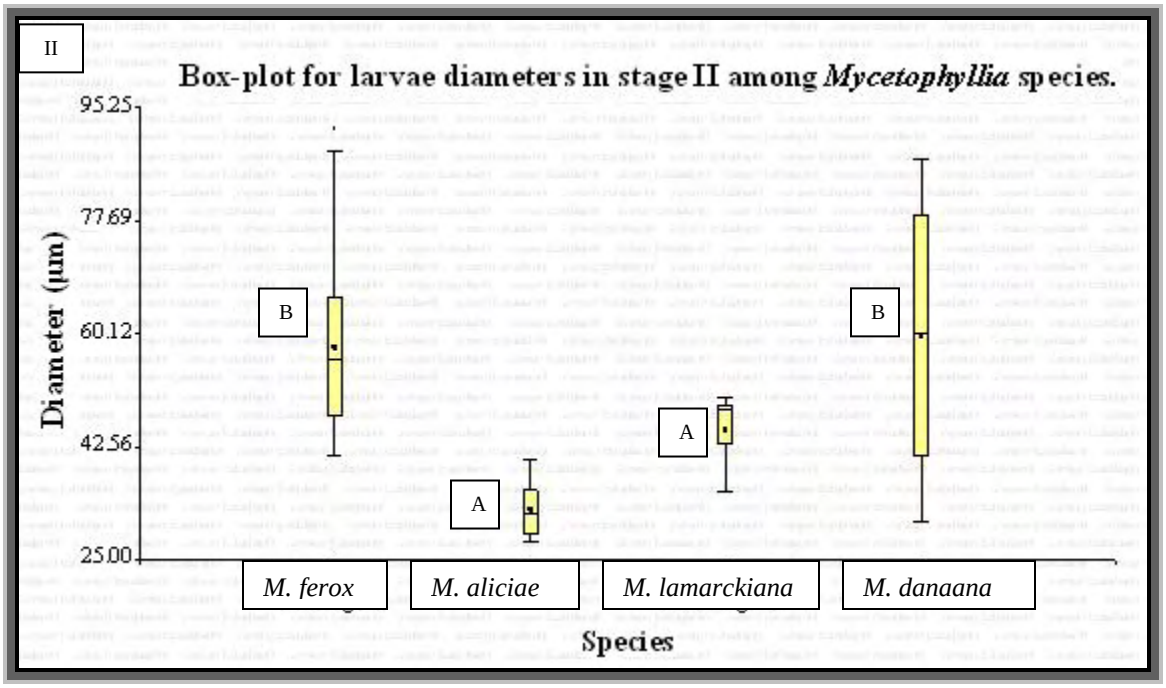
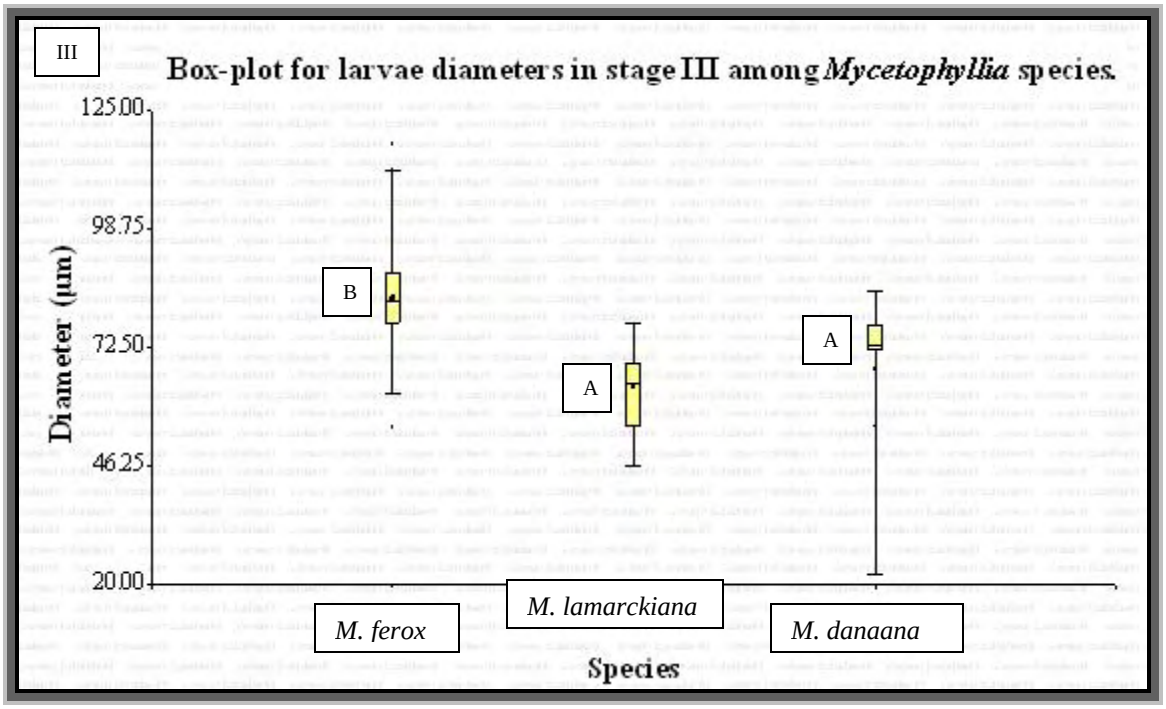


Figure 34. Box-plot comparing larvae average diameters by stage among *Mycetophyllia* species. Box-plot I show the difference in stage I among the species; Box-plot II shows the differences in stage II among the species. Y axis diameters measurements (µm) X axis developmental stages I-IV. Different capital letter (A-B) indicate significant difference among the stages.



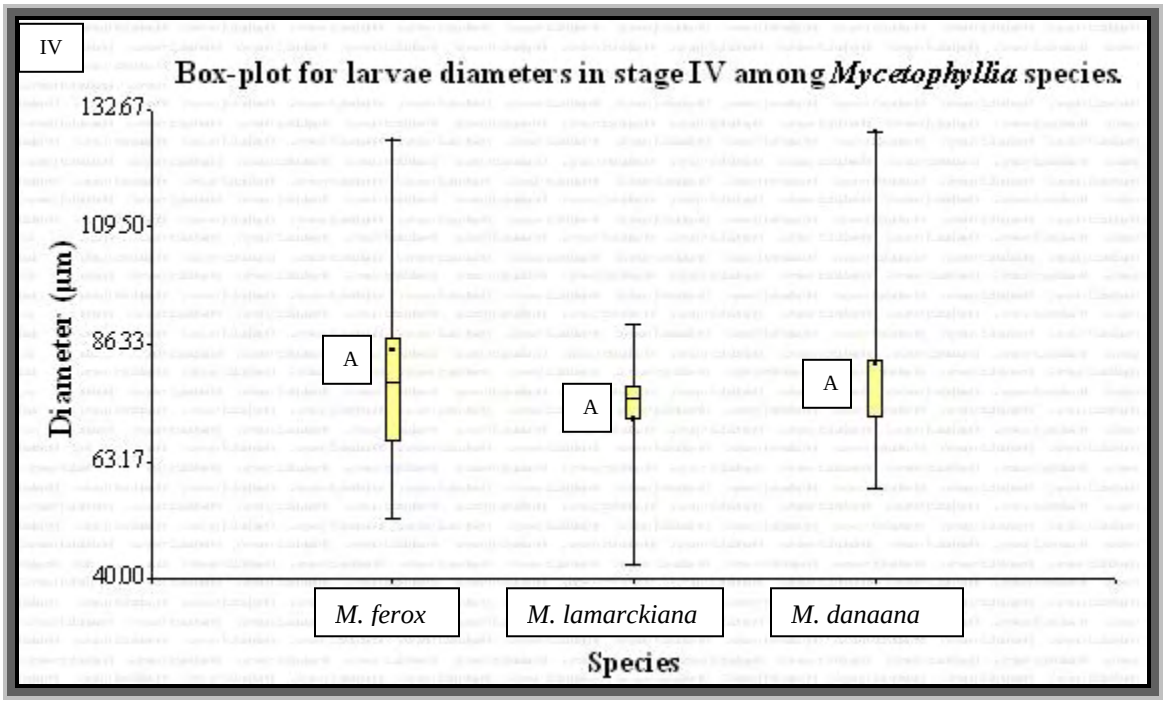


Figure 34 . Continuation: Box-plot III shows the differences in stage III among the species; Box-plot IV shows the differences in stage IV among the species. Y axis diameters measurements (µm) X axis developmental stages I-IV. Different capital letters (A-B) indicate significant difference among the stages.

C. Fecundity

Mycetophyllia species have 24 mesenteries per polyp, but not all mesenteries were used in sexual reproduction. The average number of mesenteries (number of mesenteries with gametes and embryos in stage IV/ total number of samples) used in gamete and embryo production in *M. aliciae* was 2.74 (± 2.94), in *M. danaana* 3.00 (± 2.55), in *M. lamarckiana* 3.00 (± 2.24) and in *M. ferox* 1.6 (± 1.40) (Table 19). The average number of oocytes ((Number of oocytes in stage IV/ number of mesenteries)/ total number of samples with oocytes in stage IV) produced per mesentery in *M. aliciae* was 6.36 (± 3.72), in *M. danaana* 4.56 (± 2.49), in *M. lamarckiana* 4.13 (± 3.00) and in *M. ferox* 1.25 (± 0.38) (Table 19). The average number of larvae ((Number of larvae in stage IV/ number of mesenteries)/ total number of samples with larvae in stage IV) produced per mesentery in *M. aliciae* was 1.10 (±0.14), in *M. danaana* 1.05 (± 0.14), in *M. lamarckiana* 1.03 (± 0.11) and in *M. ferox* 1.04 (± 0.12) (Table 19).

The fecundity per polyp (total of eggs in stage IV + total of larvae in stage IV) in *M. danaana* was 30.68 (± 120.27), in *M. aliciae* 25.81 (± 25.35), in *M. lamarckiana* 14.43 (± 16.55) and in *M. ferox* 3 (± 1.83) (Table 19). Nonparametric one-way ANOVA test (Kruskal-Wallis) detected significant differences ($p < 0.0001$) in polyp fecundity. *Mycetophyllia ferox* had significantly lower fecundity with respect to *M. aliciae* and *M. danaana* (Table 21; Figure 35). There were no differences between *M. aliciae* and *M. danaana* and between *M. lamarckiana* and *M. danaana* (Table 21; Figure 35). *Mycetophyllia aliciae* and *M. danaana* had the higher polyp fecundity (Table 21; Figure 35). The average fecundity per polyp estimated with the samples from November 2004 for *M. aliciae* and *M. ferox* was 46.29 and 4.13 respectively (Table 20).

The average fecundity per mesentery ((oocytes in stage IV + larvae in stage IV)/ mesenteries) in *M. aliciae* was 6.21 (± 3.75), for *M.danaana* 4.15 (± 2.57), in *M. lamarckiana* 3.59 (± 2.92) and in *M. ferox* 1.16 (± 0.31) (Table 19). Nonparametric one-way ANOVA test (Kruskal-Wallis) ($p < 0.0001$) detected significant differences in mesenterial fecundity among *M. ferox, M. aliciae* and *M. lamarckiana* (Table 22; Figure 36). There were no significant differences between *M. lamarckiana* and *M. danaana* (Table 22; Figure 36). *Mycetophyllia ferox* had the lowest mesenterial fecundity and *M. aliciae* had the higher mesenterial fecundity (Table 22; Figure 36). The average fecundity per mesentery estimated with the samples from November 2004 for *M. aliciae* and *M. ferox* was 4.57 and 0.87 respectively (Table 20).

Table 19. Estimates of average annual polyp and mesenterial fecundity for *Mycetophyllia* species in La Parguera, Puerto Rico.

Mycetophyllia	***ferox***	***aliciae***	***lamarckiana***	***danaana***
Polyps per cm²	2	1	1	1
Mesenteries/polyp	24	24	24	24
Mesenteries used in reproduction	1.6 ± 1.40	2.74 ± 2.94	3.00 ± 2.94	3.00 ± 2.55
Eggs/mesentery Average ± SD	1.25 ± 0.38	6.36 ± 3.72	4.13 ± 3.00	4.56 ± 2.49
Larvae/mesentery Average ± SD	1.04 ± 0.12	1.10 ± 0.14	1.03 ± 0.11	1.05 ± 0.14
Fecundity per polyp (# total oocytes + # total larvae) ± SD	2.56 ± 1.83	25.81 ± 25.35	14.43 ± 16.55	30.68 ± 120.27
Fecundity per mesentery (# oocyte + # larvae)/ # mesenteries ± SD	1.16 ± 0.31	6.21 ± 3.75	3.59 ± 2.92	4.15 ± 2.57

Table 20. Estimates of average polyp and mesenterial fecundity for *M. ferox* and *M. aliciae* in November 2004, in La Parguera, Puerto Rico.

Mycetophyllia	*ferox*	*aliciae*
Fecundity per polyp (# total oocytes + # total larvae) ± SD	4.13 ± 5.67	46.29 ± 37.42
Fecundity per mesentery (# oocyte + # larvae)/ #mesenteries ± SD	0.87 ± 0.77	4.57 ± 2.00

Table 21. Non parametric one-way ANOVA test (Kruskal Wallis) polyp fecundity among *Mycetophyllia* species. Different capital letters indicate significant differences ($p < 0.0001$).

Variable	species	N	Average	SD	Median	
Fecundity	*M. ali*	63	25.81	25.35	16.00	gl= 3
Fecundity	*M. dan*	63	30.68	120.27	11.00	C= 0.99
Fecundity	*M. fer*	55	2.56	1.83	2.00	H= 80.86
Fecundity	*M. lam*	65	14.43	16.55	8.00	p< 0.0001

Trat.	Ranks			
M. ferox	51.85	A		
M. lamarckiana	125.19		B	
M. danaana	144.83		B	C
M. aliciae	162.98			C

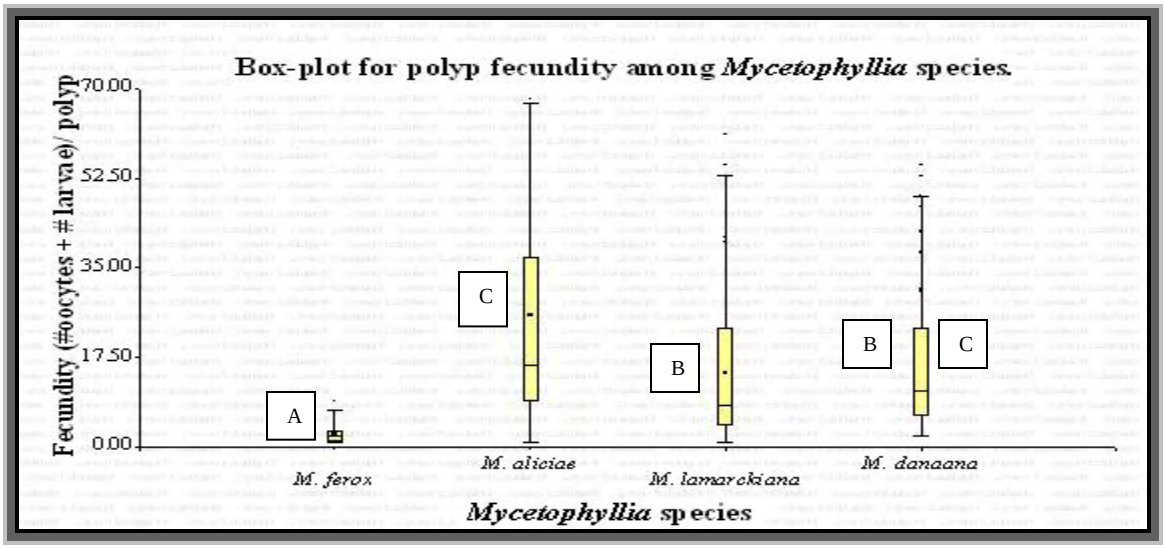


Figure 35. Box-plot for polyp fecundity among *Mycetophyllia* species. Y axis average fecundity measurements, X axis Mycetophyllia species. Different capital letters (A-B) indicate significant difference among the stages.

Table 22. Non parametric one-way ANOVA test (Kruskal Wallis) for mesenterial fecundity among *Mycetophyllia* species. Different capital letters indicate Significant differences ($p < 0.0001$).

Variable	species	N	Average	SD	Median	
mes fecundity	*M. ali*	63	6.21	3.75	5.80	gl= 3 3
mes fecundity	*M. dan*	63	4.15	2.57	4.00	C= 0.97
mes fecundity	*M. fer*	55	1.16	0.31	1.00	H= 85.06
mes fecundity	*M. lam*	65	3.59	2.92	2.50	p< 0.0001

Trat.	Ranks			
M. ferox	52.05	A		
M. lamarckiana	122.42		B	
M. danaana	141.75		B	
M. aliciae	168.73			C

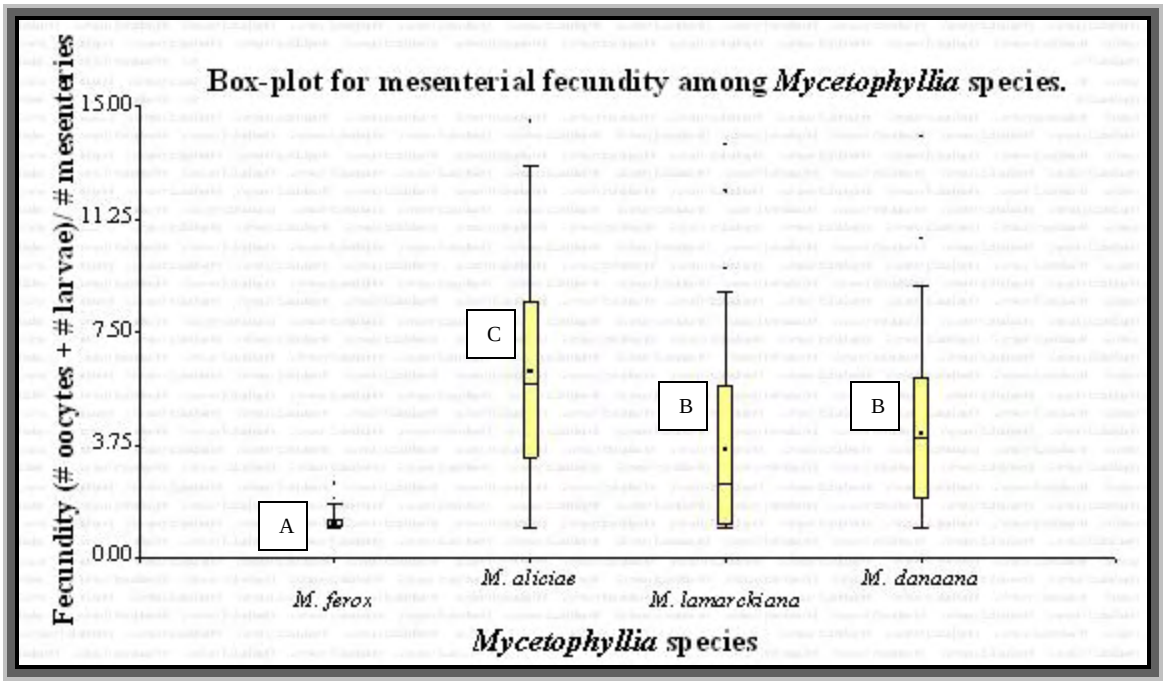


Figure 36. Box-plot for mesenterial fecundity among *Mycetophyllia* species. Y axis average fecundity measurements, X axis Mycetophyllia species. Different capital letters (A-C) indicate significant difference among the stages.

D. Environmental variables

D.1. Moon cycles

Laboratory observations (Table 9) indicated that *Mycetophyllia* species brooded during periods that include different moon phases. Histological analysis also indicated that oocytes and spermatocytes matured during different moon phases.

Mycetophyllia ferox had mature spermatocytes from December (reaching the final stage V during full moon phase) through January (new moon phase) (Figure 37). Oocyte development initiated in August (reaching stage IV during full moon phase) and ended in January (new moon phase). This period lasted five months and all moon phases were represented (Figure 37), however oocyte and spermatocyte maturation initiated in synchrony with full moon.

Mycetophyllia aliciae had mature spermatocytes from February (reaching the final stage V during first quart phase) through April (new moon phase). This period lasted three months and all moon phases were represented (Figure 38). Oocytes showed the same maturation period as spermatocytes (Figure 38). Gamete maturation was not synchronized with the full moon.

Mycetophyllia lamarckiana had mature spermatocytes from January (reaching the final stage V during first quart phase) through July (full moon phase). This period lasted 6 months and all moon phases were represented (Figure 39). Oocytes had the same maturation period as spermatocytes (Figure 39). Like *M. aliciae*, gamete maturation was not synchronized with the full moon.

Mycetophyllia danaana had mature spermatocytes during two periods. The first period was from December (reaching the final stage V during full moon phase); through February (full moon phase); this period lasted 3 months and only full moon and new moon phases were represented (Figure 40). The second period was from April (reaching the final stage V during last quart phase) through May (first quart phase); this period lasted 1 month (Figure 40). The period of oocyte maturation initiated in February (reaching the final stage IV during first quart moon phase) through March (full moon phase); this period lasted 1 month, with only first quart and full moon phases represented (Figure 40). Spermatocyte maturation was synchronized with the full moon phase, but oocyte maturation was not.

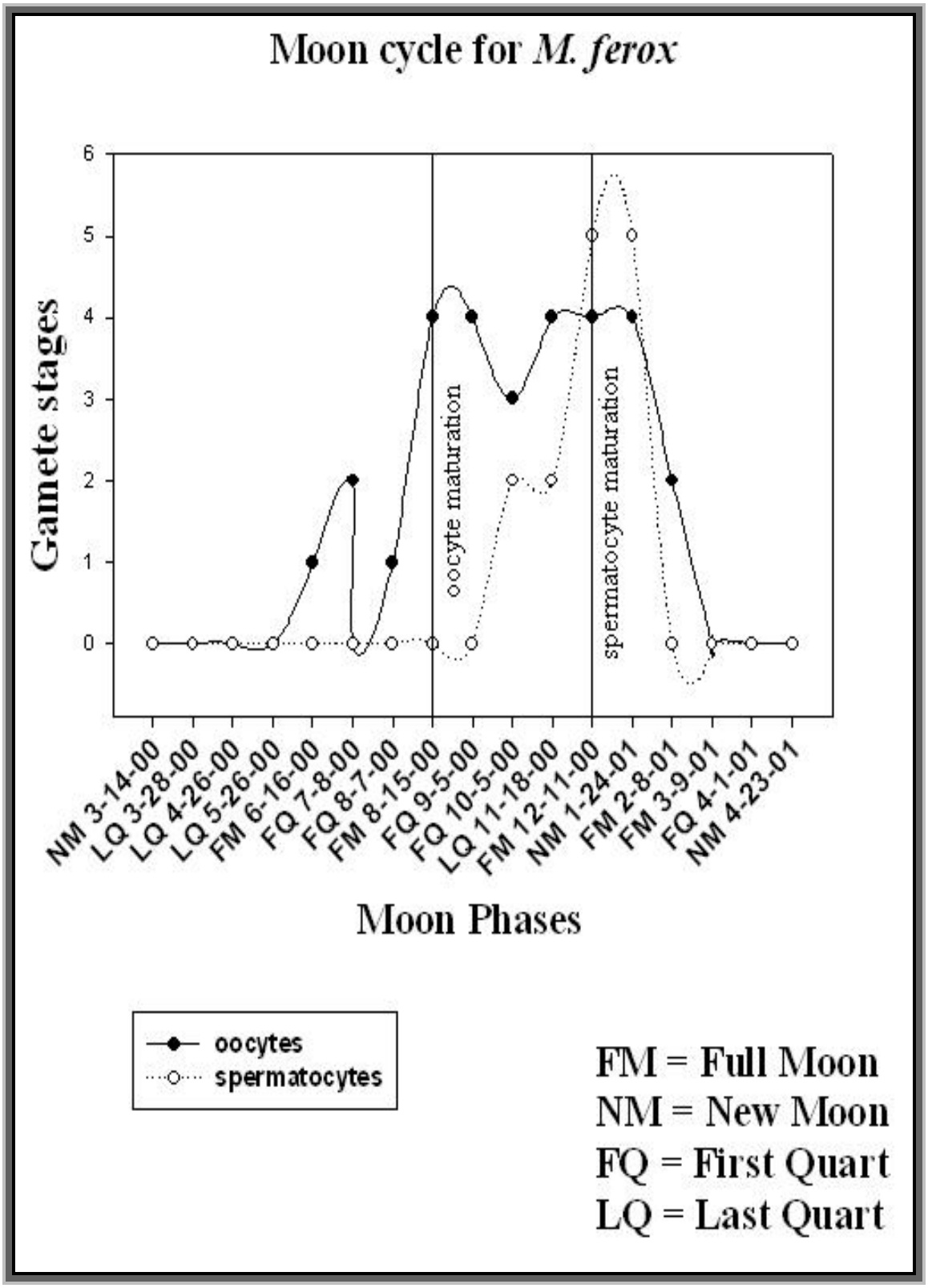


Figure 37. Periods of maturation stages of oocytes (4) and spermatocytes (5) compared with moon phases in *M. ferox*. 4= stage IV in oocytes; 5= stage V in spermatocytes.

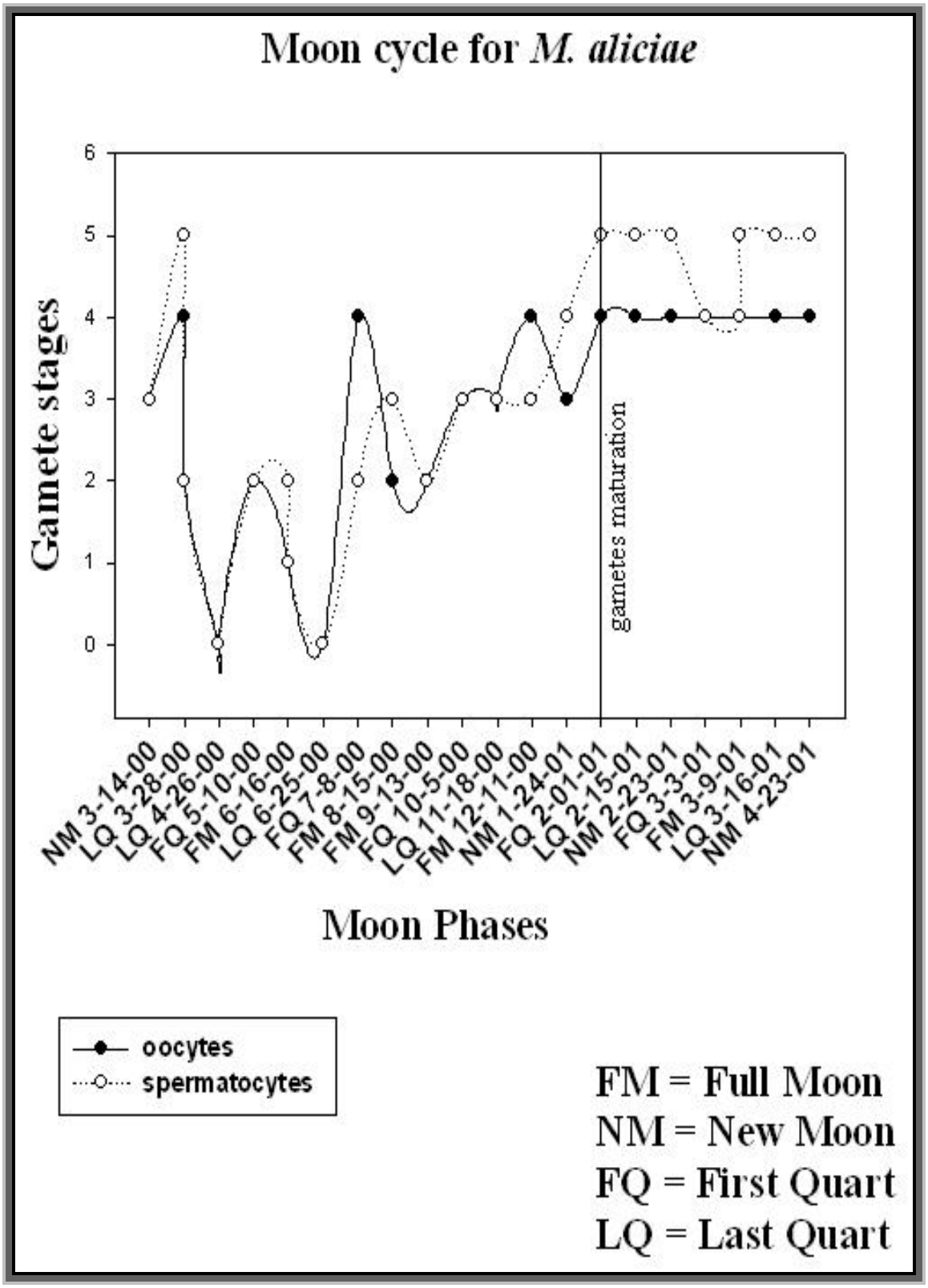


Figure 38. Periods of maturation stages of oocytes (4) and spermatocytes (5) compared with moon phases in *M. aliciae*. 4= stage IV in oocytes; 5= stage V in spermatocytes.

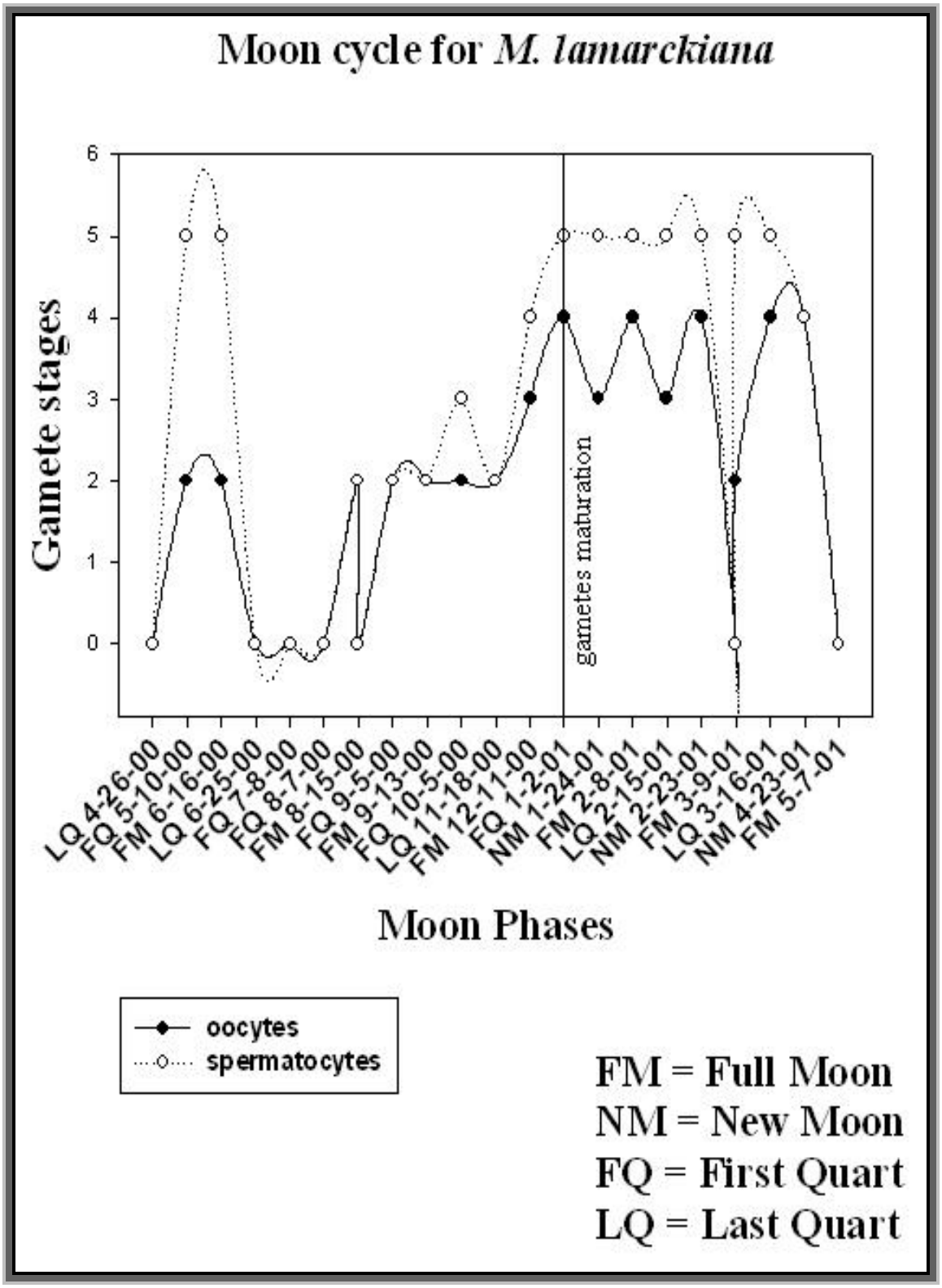


Figure 39. Periods of maturation stages of oocytes (4) and spermatocytes (5) compared with moon phases in *M. lamarckiana*. 4= stage IV in oocytes; 5= stage V in spermatocytes.

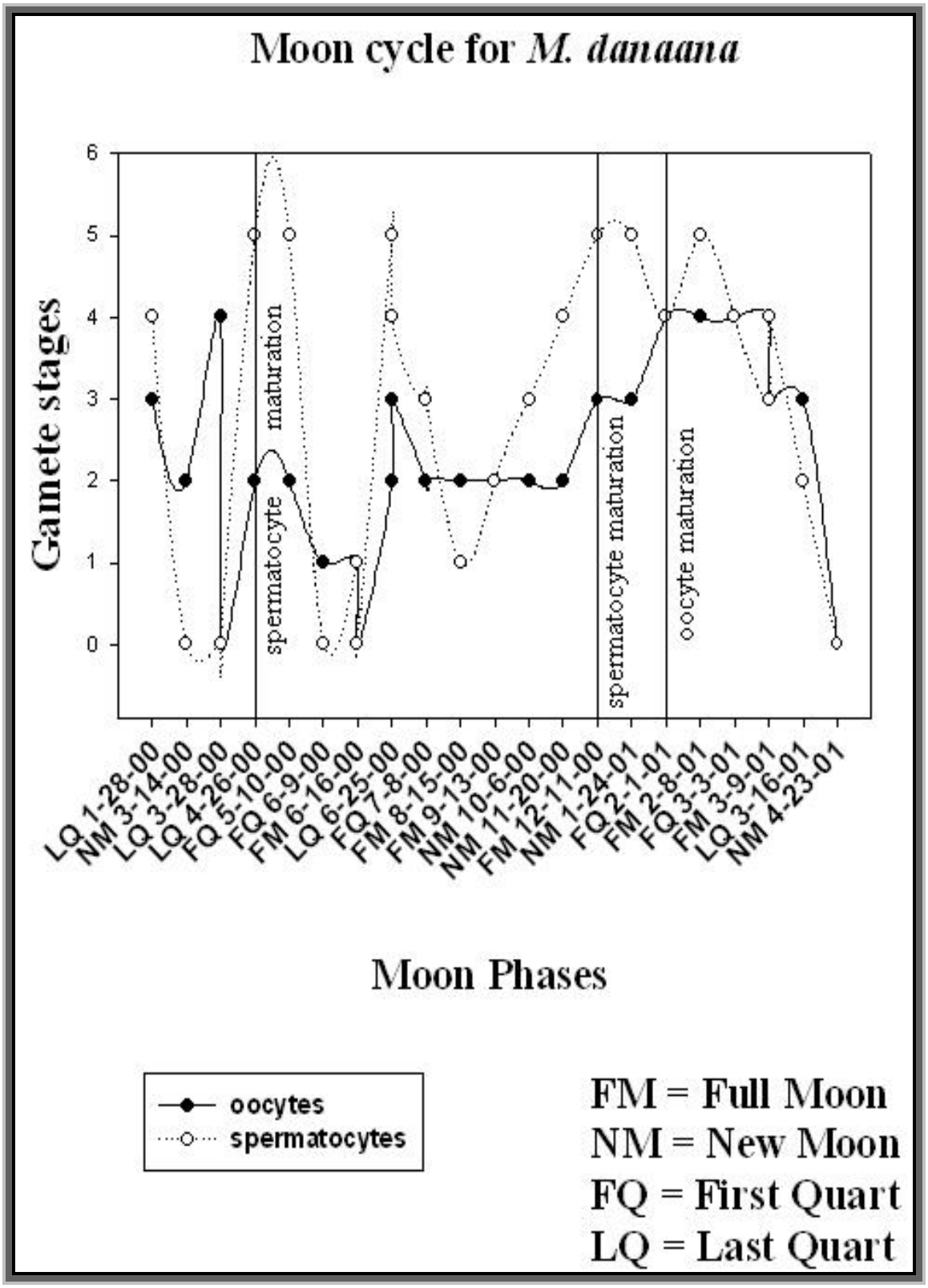


Figure 40. Periods of maturation stages of oocytes (4) and spermatocytes (5) compared with moon phases in *M. danaana*. 4= stage IV in oocytes; 5= stage V in spermatocytes.

D.2. Temperature

Surface water temperature from January 1, 2000 to December 31, 2001 varied between 25.8 °C to 30.0 °C, with an average of 28.6 °C. This web-posted chart (Figure 41) showed sea surface temperature (SST) time series at a representative satellite pixel location for Puerto Rico coral reefs. The time series is extracted from NOAA/NASA AVHRR Oceans Pathfinder daily 9 km nighttime sea surface temperature dataset.

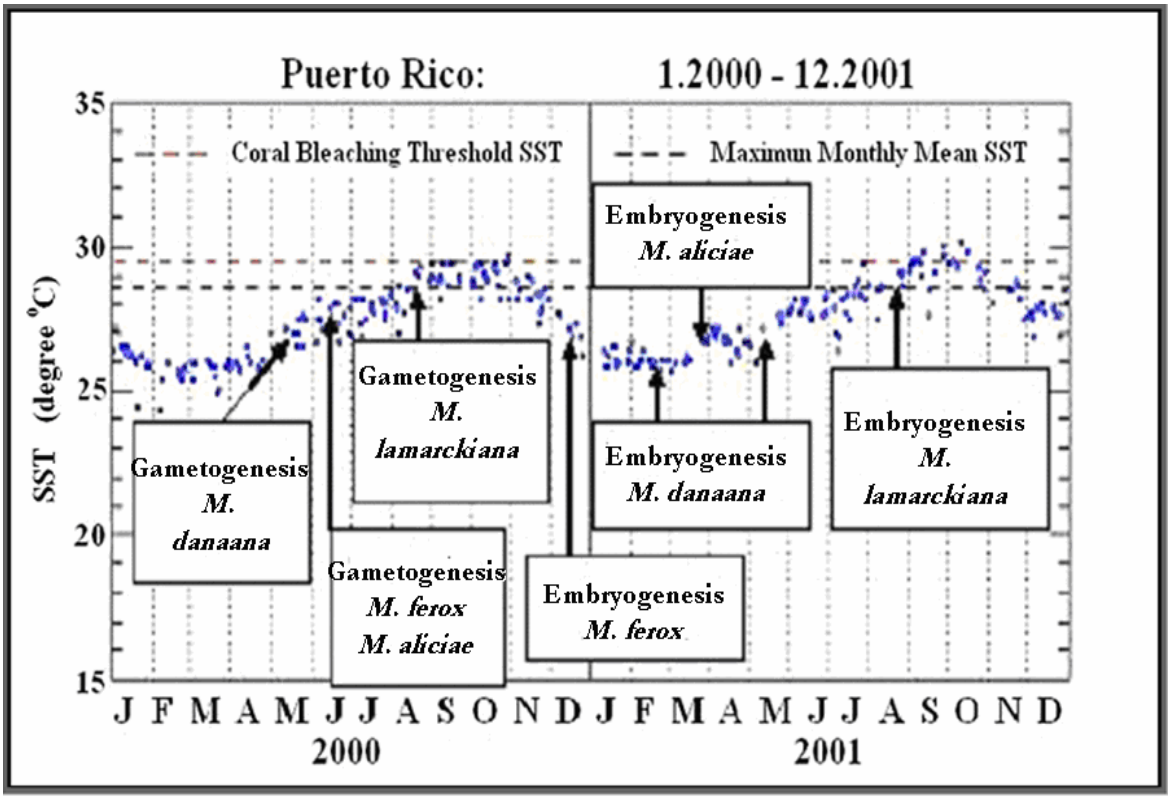


Figure 41. Puerto Rico Sea Surface Temperature Time Series Charts - Pathfinder, NOAA/NASA AVHRR Oceans SST (SST = Sea Surface Temperature), and the onset of oogenesis and embryogenesis in the four species of *Mycetophyllia.*

Temperatures descended from November to March, when they rose again. *Mycetophyllia ferox* initiated oocyte development in June (27.1 °C), *M. lamarckiana* in

August (29.0 °C), *M. aliciae* in June (27.1 °C) and *M. danaana* in May (26.5 °C) (Figure 41). The brooding season for *M. ferox* lasted from December to April, when temperature descended from 27.1 ºC to 25.0 ºC. *M. aliciae* initiated the brooding period in March with 26.5 ºC, while *M. lamarckiana* initiated in August, when temperatures where warm (28.9 ºC), *Mycetophyllia danaana* initiated the first brooding period in February (25.8 °C) and the second in May (26.7 °C) when temperatures rose again (Figure 41). These observations showed a relationship of gametogenesis and embryogenesis with high and low temperatures respectively.

D.3. Light

Histological results indicated that the oogenesis cycle in *Mycetophyllia* species initiated during the summer, under long day conditions (Figure 42). On the other hand, *M. ferox, M. aliciae,* and *M. danaana* reached the final stage of gamete maturation (IV-V) and initiated planula development during winter, under short day conditions (Figure 42). *Mycetophyllia lamarckiana* reached the final stage of gamete maturation (IV-V) in January, initiated embryogenesis in August (when the day is longer), and planula development occurred in February (Figure 42). *Mycetophyllia ferox* and *M. aliciae* initiated oogenesis in June, with sunlight periods of 13.4 h and moonlight periods of 10.6 to 12.8 h (Figure 42). *Mycetophyllia danaana* initiated gametogenesis in May, when sunlight lasted 12.9 h and moonlight lasted about 12.4 h, and *M. lamarckiana* initiated oogenesis and embryogenesis in August, when the sunlight lasted 13.3 to 12.7 h, and moonlight lasted 10.9 to 11.6 h (Figure 42).

The winter solstice occurs in December 22, with shortest day light period, of 11 h of sunlight (Figure 42). In the period characterized by short days (November-January), *M. ferox* reached the highest stages of gamete development and initiated embryogenesis. *Mycetophyllia danaana* had two periods of final stage of gamete development and the initiation of planula development. The first period initiated in February, when sunlight lasted 11.3 to 11.7 h and moonlight lasted 11.4 to 12.5 h. The second period occurred in May, when both sun and moonlight lasted about 11.75 h. In March, *M. aliciae* reached stages IV and V of gamete development and initiated embryogenesis, when the sunlight lasted 11.6 to 12.0 h and moonlight period was 12.5 h. In summary all the four species regulated gametes development with sunlight and embryo development with moonlight.

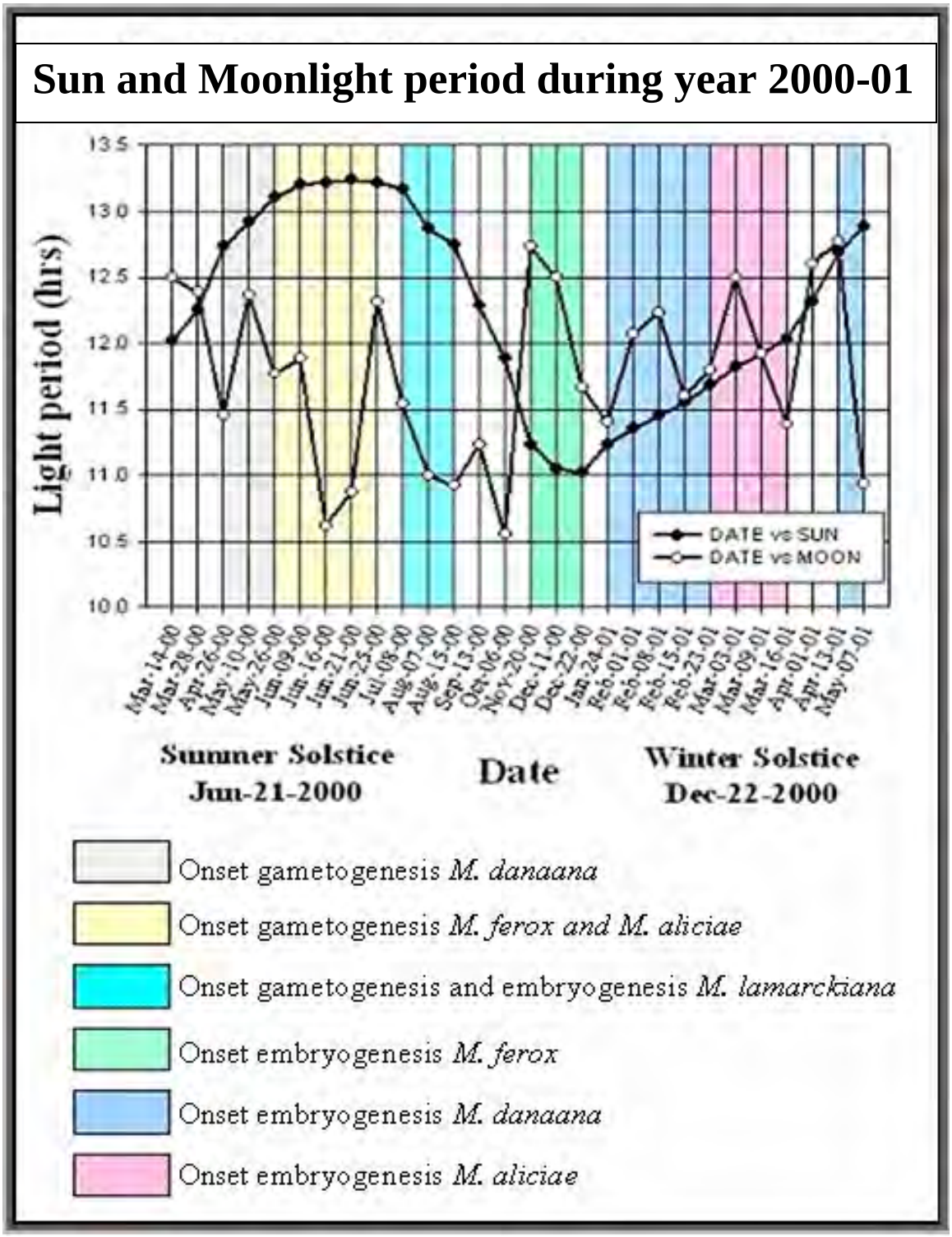


Figure 42. Sun and moonlight period during years 2000-01. Y axis shows the time period in hours between sunrise and sunset, and the time period in hours between moonrise and moonset. X axis shows the dates of sampling and the onset of oogenesis and embryogenesis in different colors.

VI. DISCUSSION

Sexual reproduction is the most important process in the life history of organisms because it generates genetic variability which is important for surviving in a changing environment. In corals it involves the processes of gametogenesis, fecundation, embryogenesis, spawning, dispersion and settlement. At present, there is an increased interest in the reproductive patterns and characteristics of most Caribbean coral species because the current trends in reef deterioration (Morelock, 2001), the increase importance of resources managing and because most of the information available is usually incomplete or conflictive (Vargas, 2002).

A. Sexual pattern and mode of development

Results from this study corroborated previous observations of the reproductive pattern and mode of development for *M. ferox* in Puerto Rico (Szmant-Froelich et al. 1985) and added new information on the reproductive biology of the other three species of this genus common to Puerto Rico and the Caribbean. *Mycetophyllia ferox* is a simultaneous hermaphroditic that initiates oogenesis before spermatogenesis, has internal development of larvae, and broods during the winter. *Mycetophyllia aliciae, M. lamarckiana* and *M. danaana* were also simultaneous-hermaphrodites. Hermaphroditism is a conservative feature of sexual reproduction in corals; it has a phylogenetic basis (Harrison, 1985), and predominates in the families Acroporiidae, Faviidae, Merulinidae, Musiidae, Pectiniidae and Pocilloporidae (Harrison and Wallace, 1990; Willis et al., 1985). There are exceptions to this rule in coral sexuality, because *Astrangia,*

Montastraea and *Porites* have species with contrasting sexual patterns, for example *A. danae* (Szmant-Froelich et al., 1980), *M. cavernosa* (Wyers et al., 1991), *P. porites* (Tomascik and Sander, 1987) and *P. furcata* (Soong, 1991) are gonochoric; while *A. solitaria* (Vaughan, 1910), *M. franksi*, *M. faveolata* and *M. annularis* (Knowlton et al., 1997; Szmant et al., 1997) and *P. astreoides* (Soong, 1991) are hermaphroditic. Hermaphroditism is the dominant condition among scleractinian corals and, it has been proposed the ancestral condition, with gonochorism being a derived character (Szmant, 1986).

The four species of *Mycetophyllia* studied were hermaphrodites and showed internal embryonic development (brooders). Advantages of being a brooder include bypassing the high gamete and larvae mortality associated with developmental stages in the water column, and increasing the chances of successful settlement on appropriate substrate (Szmant, 1986). Moreover, it has been suggested (Diekmann et al., 2002) that the brooding reproductive mode may influence the acquisition of endosymbiotic algae (zooxanthellae), which are intimately involved in nutrition and calcification of many cnidarians.

B. Gametogenesis and breeding season

Gametes developed first within the mesoglea lining, then migrated into the mesenteries. Different stages of development occurred simultaneously within individual gonads throughout most of the gametogenetic cycle, with maturation reached during winter. The syngonic and digonic (both patterns intermingled) distribution of oocytes and spermatocytes within the mesenteries was consistent in the four species. This

organization of gametes suggests that these corals control either the migration of primordial germ cells of each sex, or the differentiation of germ cells within each mesentery (Harrison, 1985). The different arrangements of gametes suggest that these species may self-fertilize and cross-fertilize during the same breeding season. Brooding is closely associated with an opportunistic-type life history. Therefore, it would be advantageous for these brooders to be able to achieve a reasonable success of reproduction with self-fertilization (Szmant, 1986). The ability to self-fertilize could assure individual colonies some chance of passing on their genome to future generations (Williams, 1975; Baker, 1959).

Histological analysis suggests that cross-fertilization could be the major strategy among *Mycetophyllia* species. However, self-fertilization could be occurring under particular conditions only. *Mycetophyllia ferox* may be favoring cross-fertilization through temporal separation of gamete final maturation for littler time (may be hours or days) in the final stage of development. During the same breeding season, this species initiated gamete maturation at different times (oocytes in stage IV from August to January and spermatocytes in stage V from December to January). This difference in the onset and rate of final stage development of gametes among different colonies, would allow a colony with mature spermatocytes and digonic arrangement of gametes to fertilize a colony with immature spermatocytes but with mature oocytes. Such a species would also promote cross-fertilization with self-recognition mechanisms to distinguish sibling gametes from gametes of other colonies (Heyward and Babcock, 1986). Another way to favor cross-fertilization is through spatial separation of gametes, developing gametes with basal (oocytes) and distal (spermatocytes) positions within the same

mesentery (thus reducing the possibility of self-fertilization - Szmant-Froelich et al., 1985) or in different mesenteries. However, self-fertilization could occur in *M. ferox* because from December to January histological samples showed oocytes in stage (IV) and spermatocytes in stage (V) simultaneously with syngonic arrangement within the same mesenteries.

Mycetophyllia danaana, like *M. ferox*, could avoid self-fertilization using temporal separation of matured gametes for a brief period, with spermatocytes maturing first (December to March) followed by oocytes; developing oocytes from February to May and spermatocytes again from April to June. This difference in the onset and rate of final stage of gamete development among different colonies could allow the release of mature spermatocytes by a colony before reaching oocyte maturation; the released spermatocytes would thus fertilize another colony with matured oocytes. Also, *M. danaana* species could promote cross-fertilization with space separation of gametes, developing oocytes in basal position and spermatocytes in distal position within the same mesentery (Szmant-Froelich et al., 1985) and developing gametes in different mesenteries. Like *M. ferox*, this species would increase cross-fertilization with self-recognition mechanisms (Heyward and Babcock (1986). However, self-fertilization could occur in *M. danaana* because histological samples for February and March had oocytes in stage (IV) and spermatocytes in stage (V) simultaneously with syngonic arrangement within the same mesenteries. Thus, the polyps could fertilize their own oocytes with spermatocytes produced during the second spermatogenesis cycle (April to June).

On the other hand, self-fertilization could be the major strategy in *M. aliciae* and *M. lamarckiana* because they have oocytes in stage IV and spermatocytes in stage V

simultaneously within the same mesentery. In these species, there is neither spatial nor temporal separation between male and female gametocytes, and thus self-fertilization could occur. However, cross-fertilization could also occur because both species also showed spatial separation of gametes, developing oocytes in basal, and spermatocytes in distal positions within the same mesentery, and developing gametes in different mesenteries. A genetic analysis of the larvae and parental colonies are need to corroborate these observations. Histological observations in *Balanophylia europea* showed that like, *Mycetophyllia* species, there is neither spatial nor temporal separation between male and female gametocytes, and that encounters occurred between mature spermatozoa and oocytes produced by the same individual. The observations in *B. europea* strongly supports the hypothesis that self-fertilization characterizes the reproductive biology of *B. europea* (Goffredo and Telò, 2000; Goffredo et al., 2002).

The genetic structure of the self-fertilizing, brooding coral *Acropora palifera*, which was studied using allozymatic loci, exhibited a deficit of heterozygotes similar to that found in *B. europea* (Carlon, 1999). Self-fertilization is associated with poor mobility or a sessile life style, low population density (Tomlinson 1966; Kojis and Quinn, 1981), and/or colonization of disturbed habitats that could limit cross-fertilization (Bucklin et al., 1984). Szmant 1986 also suggested that while self-fertilization is common in brooders, for the same reasons selfing is advantageous in higher plants, a considerable effort is made to increase out-crossing by some species. Brooders such as *Favia fragum* and *M. ferox* had large numbers of spermaries that often outnumbered the eggs in number and in volume by a factor of five to ten (Szmant, 1986). This would appear to be extremely wasteful if self- fertilization was common (Szmant, 1986).

Hermaphroditism coupled with brooding is generally correlated with small colony size, high recruitment and many gametogenetic cycles per year, as occurs in Caribbean species such as *Porites astreoides,* which has 9 gametogenetic cycles per year (Szmant, 1986), and *F. fragum,* which has 12 gametogenetic cycles per year (Szmant-Froelich, 1985). *Favia fragum* and *P. astreoides* release their planulae at regular intervals in phase with the lunar cycle (Szmant-Froelich, 1985). Different to these species in the Caribbean, *Mycetophyllia* species had only one gametogenetic cycle during the year and one extended brooding season (Szmant 1986). *Mycetophyllia* species showed different periods of planulation. *Mycetophyllia ferox* like, *P. porites,* (Tomascik and Sander, 1987) brooded during winter. *Mycetophyllia aliciae* and *M. lamarckiana* brooded during spring, like *Agaricia agaricites* (Duerden, 1902; Van Moorsel, 1983), *A. crassa* (Vaughan, 1910) and *Isophyllia* sp. (Duerden, 1902). *Mycetophyllia danaana* brooded year around as *A. humilis* (Van Morsel, 1983, *F. fragum* (Szmant, 1986), *Manicina areolata* (Fadlallah, 1983), *P. astreoides* (Szmant, 1986), and *Siderastrea radians* (Szmant, 1986). Different to those species, *Manicina areolata* (Fadlallah, 1983) and *A. frajilis* (Mavor, 1915) brooded during summer.

C. Planula development and brooding season

Brooded planulae develop slower that non-brooded larvae, and may take weeks or months to complete its development (De Lacaze-Duthiers, 1873; Szmant-Froelich, et al., 1985; Stoddart and Black, 1985; Kojis, 1986b). *Balanophyllia. elegans* planulae has an extremely prolonged brooding period, lasting 14 or 15 months (Fadlallah and Pearse,

1982a). Brooded planulae often increase greatly in size during development, and may grow to fill much of the available space within the polyp coelenteron (Delvoye, 1982; Fadlallah and Pearse, 1982a; Szmant-Froelich et al., 1985; Kojis, 1986a; Szmant, 1986). Most scleractinian planulae have similar morphological and behavioral characteristics regardless of whether they are brooded or developed externally; sexually or asexually derived. They are generally described as barrel-shaped, cigar-shaped, spheroidal, pyriform, or elongated and are capable of changing shape rapidly.

Development of larvae in *M. ferox* occurred from December until April, when larvae reached stage IV; this was the species that showed well developed larger larvae. *Mycetophyllia aliciae* differed from the rest of the species studied because the highest stage of planula development observed was stage II, from March to April. A possible explanation for this difference may be the lack of space for the high number of gametes produced and the increase of dispersion range by finishing development in the water column. Embryo development in the gastrula stage was only observed in *M. lamarckiana* from August through January. In February, larvae changed to stage II and developed quickly to stage IV by May. *Mycetophyllia danaana* had one extended period of planula development throughout the year, with a maturation peak in April. This brooding period may result from two spermatogenesis cycles. The period of brooding initiated in May and ended in April, during the winter. This species showed low percentages of larvae of stage II in July and of stage III in October, suggesting that it could release larvae in different stages during the year before reaching the final stage IV. Planulation for these four species was observed in colonies under laboratory conditions from December to March

2000 by Dr. Weil (Department of Marine Sciences of the University of Puerto Rico, in La Parguera). His observations agree with the histological analysis presented in this work.

D. Environmental factors regulating sexual reproduction

Temperature data for this research was compared with the temperatures registered by Vargas (2002) in her study of sexual reproduction in the Caribbean coral genus *Diploria,* in La Parguera, Puerto Rico. In her study, temperatures ranged from 25.5 °C to 31.0 °C during 1999-2000. This range was similar to the range of temperature obtained from NOAA/NASA AVHRR Oceans Pathfinder daily 9km nighttime sea surface temperature dataset for this study. Water temperature increase seem to be an important factor regulating the onset of gametogenesis in all species of *Mycetophyllia* (Figure 41). *Mycetophyllia ferox, M. aliciae* and *M. lamarckiana* initiated gametogenesis during the summer (June to August), while *M. danaana* initiated gametogenesis during late spring, when sea surface temperatures ranged from 26.5 to 28.7 ºC. The gametes of *M. ferox, M. danaana* and *M. aliciae* reached the final stages of development and continued with embryogenesis when sea surface temperatures began to descend. Embryogenesis in *M. lamarckiana* could be regulated by temperatures reached higher values (coral bleach at 29.0 ºC) and gastrula development was slow. After February, planulae changed faster from stage II to stage IV, suggesting that optimal temperatures for planula development in *M. lamarckiana* occured during winter (~ 25.0°C).

Seasonal variation in sea temperature has been widely accepted as the most important environmental factor controlling gametogenetic cycles or planula-release periods in scleractinian corals (Babcock et al., 1986). Differences in the timing of gamete

maturation and spawning, between populations of the same coral species on inshore and offshore reefs in the central Great Barrier Reef, and corresponding to differences in the time of rise in sea temperatures at these sites, provide indirect evidence for the influence of temperature on gamete maturation in corals (Harrison et al., 1984a; Babcock et al., 1986). Initiation and development of oocytes were similar among populations of three faviid species studied on inshore and offshore reefs in this region, but testes development, gamete maturation, and spawning occurred one month earlier on the inshore reef, coinciding with an earlier and more rapid rise in temperature (Babcock, 1986). Babcock suggested that temperature may regulate the timing of spawning through controlling testes development.

Reproductive activities of many scleractinian corals are synchronized with lunar phases (Harrison and Wallace, 1990). Gamete maturation and release occurs about the time of full moon, or a few days afterwards, in *Agaricia agaricites, A. humilis, Favia fragum* and *Porites astreoides* (Delvoye, 1982; Szmant-Froelich et al., 1985; Chornesky and Peters, 1987). *Mycetophyllia* species have seasonal breeding periods during winter that include all moon phases. Gamete maturation for *M. ferox* starts with the full moon phase. Oocytes reached stage IV from October to January and spermatocytes reached stage V from December to January for the same breeding season. Like *M. ferox, M. danaana* also starts gamete maturation in synchrony with full moon phase. In December, spermatocytes reached stage V and in February oocytes reached stage IV.

On the other hand, *M. aliciae* and *M. lamarckiana* initiated gamete maturation simultaneously; both during a first quarter moon phase. These species did not show the relationship with the full moon described for scleractinian corals. It would be suggested

that moonlight and tidal cycles provide cues to synchronize reproductive cycles in species with different onset of gamete development. Species with the same onset of gamete development could regulate gamete maturation with different parameters, such as sea temperature, day light, food, and daily light/dark cycles. Periods of planula release are generally less clearly delineated than breeding periods, because planula release can occur during different stages of development (Fadlallah, 1983; Motoda, 1939; Jells, 1980; Shlesinger and Loya, 1985; Kojis, 1986a; Wright, 1986). The maturation and duration of planula-release periods varied widely among *Mycetophyllia* species, which exhibited no lunar periodicity of planula release.

Light is a proximate and ultimate factor regulating reproductive cycles. Day light, moonlight, daily light/dark cycles and, seasonal changes in day length may act differently to coordinate each stage of gamete development (Harrison and Wallace, 1990). On December 22 (winter solstice), day light is shorter than during the rest of the year and nocturnal illumination is larger. These changes in illumination in combination with the lunar phases may provide the fine tuning for particular nights of spermatocyte or planula release in *Mycetophyllia*. In the four species analyzed in this study, the reproductive cycle could be regulated by the relationship between seasonal changes in sunlight and moonlight. All *Mycetophyllia* species initiated gametogenesis from May to August, when sunlight exceed moonlight. On the other hand, *M. ferox, M. aliciae* and *M. danaana* initiated brooding after the winter solstice, when moonlight exceed sunlight. *Mycetophyllia lamarckiana* was the only species that initiated brooding in August (with latent gastrula stage), when the sunlight was larger than moonlight, but like the other *Mycetophyllia* showed more developed planulae during winter.

VII. CONCLUSIONS

- All four *Mycetophyllia* species are simultaneous-hermaphrodites that brood larvae.

- All four species showed different timing and duration in their gametogenesis and embryogenesis, a possible mechanism to prevent hybridization.

- Gamete arrangement was similar in all species, with both syngonic and digonic patterns.

- The four species had only one gametogenetic cycle a year but brood larvae during extended periods (several months).

- *M. ferox* showed the lowest mesenterial fecundity but, produced the most developed larvae, competent to settle down shortly after leaving the parental colony. This is the most abundant of all *Mycetophyllia* species.

- *M. aliciae* had significantly higher mesenterial fecundity and might liberate larvae in early stages (L-II), which could enhance the dispersion distances.

- *M. lamarckiana* and *M. danaana* had intermediate fecundities and larvae in stage IV are less developed than *M. ferox.* Both species have lower abundances than *M. ferox* and *M. aliciae*.

- There is a potential for self-fertilization in all species; Further genetic analyses of the larvae should be done.

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IX APPENDIX 1: Solutions used in laboratory procedures

A. Zenker Formalin (Helly`s Solution) for sample fixation

- Zenker formalin = 100 ml of Zenker Base + 5 ml of (37-40%) of Formaldehyde

- Zenker Base for 1L: 50g Mercuric Chloride
  25g Potassium Dichromate
  10g Sodium Sulfate
  1000 ml Distilled Water

B. 10% Hydrochloric acid (HCl) for decalcification

- 1L of 10% HCl = 900 ml of distilled water + 100 ml of 100% HCl

C. 70% of Ethanol (ETOH) for preservation

- 1L of 70% ETOH = 750 ml of 95% ETOH + 250 ml of distilled water

D. Dehydration and clearing procedure in the rotary Tissue Processor

- Dehydration initiated with a series of different concentrations of ETOH, and Isopropanol solution (Tissue Dry). Two baskets with 15 to 20 tissue capsules with samples/basket.
  - Glass container or position 1 and 2 with 70% Ethanol
  - Glass container or position 3 with 95% Ethanol
  - Glass container 4, 5 and 6 with Tissue Dry (Isopropanol solution)

- Then, samples were cleaned in xylene solution (Tissue Clear III), and finally dipped in Paraplast (Tissue Prep)
  - Glass container 7, 8 and 9 with Tissue Clear (xylene solution)
  - Container 10 and 11 with paraffin (Tissue Prep) (at 56 ºC)

- Each basket stays 1 hr in each one of the containers or position (1-11)

E. Staining procedure with Heidenhain`s Aniline-Blue Method:

- Samples were deparaffinized with xylene solution (3 glass containers with xylene solution) during 3 min interval in each container.

- Hydrated to distilled water: 3 glass containers with 100% alcohol, one with 95% alcohol, one with 70% alcohol and one container with distilled water. With 3 minutes interval at each solution.

- Samples were placed in the pre-heated Azocarmine B solution at 56 ºC for 28-30 minutes.

    - 1% Azocarmine = 3g Azocarmine B + 300 ml distilled water (boil) + 3 ml Glacial acetic acid

- Rinsed in tap water, then in distilled water for 1-3 min.

- Differentiated in Aniline-Alcohol solution for 8-10 min.

    - Aniline-Alcohol = 300 ml 90% alcohol + 3 ml Aniline

- Mordant in Phosphotungstic acid solution for 15 min

    - Phosphotungstic acid = 300 ml distilled water + 9g Phosphotungstic acid

- Rinsed in distilled water for 2 min.

- Stained with Aniline-Blue solution for 15-20 min.

    - Aniline-Blue = 300 ml distilled water + 0.5g Aniline-Blue + 2g Orange G + 8 ml Acetic acid

- Rinsed in tap water, then in distilled water for 2 min.

- Dehydrated with 70% alcohol, 95% alcohol, and 3 glass containers with 100% alcohol at 2 min interval.

- Cleared with xylene solution (3 glass containers)

- Mounted with Cytoseal