Sexual Reproduction in the Caribbean Coral Genera *Isophyllia* and *Isophyllastrea,* in La Parguera, Puerto Rico

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
IN
MARINE SCIENCES
BIOLOGICAL OCEANOGRAPHY

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2014

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ABSTRACT

The sexual pattern, reproductive mode, and timing of reproduction of *Isophyllia sinuosa* and *Isophyllia rigida*, two small Caribbean Mussids, were assessed by histological analysis of specimens collected monthly during 2000-2001 and 2012. Tissue cores were fixed in Helly's Zenker formalin solution, decalcified in 10% HCl, dehydrated in 70% EtOH, embedded in Paraplast, sectioned with a rotary microtome, and stained utilizing a modified Heidenhain's Azocarmine-Aniline blue method. Results indicate that both species are simultaneous hermaphroditic brooders, with a single annual gametogenetic cycle. Spermatocytes and oocytes of different stages were found within the same mesentery indicating possible sequential maturation for extended planulation. Oocytes begin development in May in *I. sinuosa* and August in *I. rigida,* approximately 7 months prior to spermatocytes, but matured simultaneously over at least two months; May-June in *I.rigida* and March-April in *I. sinuosa*. Significantly higher polyp (IR=33.16±4.44, IS=3.78±6.42) (t-test, $p<0.001$) and mesenterial fecundity (IR=2.76±4.44, IS=0.87±1.92) (t-test, $p=0.046$) was found in *I. rigida* compared to *I. sinuosa*. Planulation proceeded rapidly in both species with planulae observed in *I. sinuosa* during April through May and in *I. rigida* from June through September. Hermaphroditism and brooding have also been documented in the Mussid genera *Scolymia* and *Mycetophyllia*.

RESUMEN

El patrón sexual, modo reproductivo y sincronización de la reproducción se estudió en dos Musidos Caribeños, *Isophyllia sinuosa* e *Isophyllia rigida*, Se hicieron análisis histológicos de tejidos colectados mensualmente durante los años 2000-2001 y 2012. Muestras fueron fijadas en solución de formalina Helly's Zenker, decalcificadas en ácido clorhídrico 10%, deshidratadas en etanol 70%, fijadas en bloques de parafina, seccionadas en un micrótomo rotativo y teñidas utilizando la tinción modificada Heidenhain's Azocarmine Aniline Blue. Resultados indican que ambas especies son hermafroditas simultaneas y planuladoras, con un ciclo gametogénico anual. Espermarios y huevos en diferentes estadios de madurez fueron observados simultáneamente en los mesenterios, indicando un proceso de maduración secuencial para una planulación extendida. Los huevos comienzan su desarrollo unos 7 meses antes que los espermarios; mayo en *I. sinuosa* y en agosto en *I. rigida.* Ambos sexos alcanzaron su madurez simultáneamente; en mayo-junio en *I. rigida* y en marzo-abril en *I. sinuosa.* La fecundidad polipal (IR=33.16±4.44, IS=3.78±6.42) (t-test, $p<0.001$) y mesenterial (IR=2.76±4.44, IS=0.87±1.92) (t-test, $p=0.046$) fue significativamente mayor en *I. rigida* comparada con *I. sinuosa.* La maduración de plánulas procedió rápidamente en ambas especies; plánulas fueron observadas en *I. sinuosa* durante abril hasta mayo y en *I. rigida* desde junio hasta septiembre. El hermafroditismo y la planulación se han documentado en los géneros Musidos *Scolymia* y *Mycetophyllia*.

ACKNOWLEDGEMENTS

My most sincere thanks go out to Dr. Frances Crespo for being a source of strength and support.

I would like to acknowledge all those who collaborated with and supported this research. I thank Dr. Ernesto Weil for allowing me the opportunity to work within his laboratory and learn from his vast experience. I would thank Mrs. Isabel Urrieztieta for patiently teaching me how to prepare histological samples and teaching me the right way to do things.

I thank the Department of Marine Sciences of UPR for providing logistical support and its employees who were always willing to help a student in any way possible. I cannot forget all my friends and coworkers who helped me perform my field work.

Partial funding was provided by Grants from Sea Grant (R-101-1-98), NOAA Coastal Ocean Program (NA17OP2919), the Caribbean Coral Reef Institute and NSF (IOS 1017510) to Dr. Ernesto Weil.

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INTRODUCTION

Coral reefs are among the most diverse and productive ecosystems in our biosphere. Coral reefs are estimated to cover an area of 255,000 km^2 (Spalding and Greenfeld 1997) which translates to less than 0.1% of the ocean's surface area. This small surface area in turn supports approximately 25% of marine species (500,000 to 3 million species) (Reaka-Kudla 2008; Bouchet 2006). Coral reefs harbor at least 32 of the 34 extant metazoan phyla, vastly more diverse than the tropical rain forest ecosystem which supports only 9 metazoan phyla (Porter and Tougas 2001). The incredible amount of diversity present in these ecosystems represents a huge potential source of scientific, pharmaceutical and aquacultural research (Birkeland 1996). Reefs also support and replenish fisheries by partially hosting the life cycles of many commercially important fish and shellfish which are dependent on the presence of healthy mangroves, coral reefs and sea grass plains. It is estimated that 10% of world production fisheries potential is derived from coral reef fisheries (Munro 1984).

Reef productivity is high; carbon dioxide is fixated into organic matter by photosynthesis and into limestone by calcification, at a rate of approximately 700 x 10^{12} g C yr^{-1}, while net production of organic material is of the order of 20 x 10^{12} g C yr^{-1}(Crossland et al. 1991). Countless organisms rely on reef ecosystems for habitat and sustenance, either by direct access or by derivation of its natural resources. Not least amongst these are humans, hundreds of millions of who depend on the reef ecosystem for food, income and raw materials (Birkeland 1996).

Throughout geological time, coral reefs have been important shapers of their surrounding environments. Coral reefs are responsible for creating islands and entire archipelagos, as well as enlarging and extending continental coastlines, by producing limestone at a rate between 6.8 and

8.3 x 10^{11} kg per year (Montaggioni and Braithwaite 2009). The reef structure also provides protection from storms and wave damage to coastal human populations making coastlines safe for transportation, tourism, and fishing.

Though the importance of coral reefs is clear, they are currently and continually being threatened by human activities such as over-fishing, climate change, pollution and habitat degradation. The effects of these activities result in some obvious consequences such as death and diminished coral cover while other consequences, such as the occurrence and aftermaths of disease, may be more difficult to distinguish (Bruno et al. 2003). Recent studies have detected a worldwide increase in the prevalence and severity of marine diseases over the past three decades affecting important marine organisms such as mollusks, turtles, mammals, echinoderms and corals (Harvell et al. 2004).

The effects of climate change and anthropogenic stressors have been shown to negatively affect multiple aspects of coral physiology and have led to the emergence of multiple new diseases and multiple severe epizootic events (Weil 2002; Weil et al. 2009; Weil and Rogers 2011) One of the direct consequences of coral disease and the subsequent tissue loss is a significant reduction or paralysis in reproductive output if the effective reproductive size of the organism falls below a particular size threshold for viable sexual reproduction (Szmant-Froelich 1985; Szmant and Gassman 1990; Weil et al. 2009). Over time, a reduction in the reproductive output of corals may lead to severe consequences for the reef ecosystem such as phase shifts, reductions in coral cover and reduced reef biodiversity (Harvell 2004; Lesser et al. 2007; Work et al. 2008). In this manner, the ability of coral species to adapt to modern-day environmental pressures depends greatly on their ability to reproduce successfully.

While some groups of scleractinians have been more thoroughly studied than others, little is known about the reproductive patterns and characteristics of most Caribbean coral species and much of the available information is conflictive or incomplete (Weil and Vargas 2010). Currently, there is information available on sexual pattern for 416 scleractinian species and mode of development for 428 species (Harrison 2011). Of the approximately 60 Caribbean zooxanthellate coral species, good information is available on 19 species with many other studies available providing partial or conflicting results (Weil 2003; Weil and Vargas 2010).

Any ecological studies of coral populations and dynamics as well as for reef community management efforts will require thorough knowledge of coral reproductive biology as an important prerequisite. This information in combination with knowledge of taxonomy, distribution and variation of the species are essential for any coral reef management strategies (Harrison and Wallace 1990). This highlights the need for research into undescribed or poorly described species through high-quality studies. Little or no information is available on the reproductive patterns and mode of reproduction of the two Mussid species *Isophyllastrea rigida* and *Isophyllia sinuosa*. This research provides an assessment on the reproductive trait of both species through detailed histological analysis.

Coral Life Cycle

Scleractinian corals, like other cnidarians exhibit a biphasic life cycle composed of a benthic polypal stage and a planktonic larval stage. The larvae (also known as planulae) are the culmination of the sexual reproductive process through a variety of different mechanisms (see below). Planulae are released into the water column where they disperse until a suitable and

permanent location on the substrate can be selected and colonized. Metamorphosis then transforms the motile recruit into a sessile juvenile polyp. Through asexual division, a single polyp can produce an entire adult colony consisting entirely of clonal individuals. Upon reaching maturity, a colony can then reproduce by both sexual and asexual processes, thus completing the coral life cycle (Harrison and Wallace 1990).

Sexual and Asexual Reproduction

Reproduction in corals consists of a sequence of events that include: gametogenesis, spawning, fertilization, embryogenesis and recruitment (Harrison and Wallace 1990). The success of the reproductive effort is determined largely on the timing, duration, frequency and intensity of the aforementioned events (Babcock et al. 1986). In corals, sexual pattern, mode of reproduction, fertilization, larval dispersal, recruitment and survivorship are key components in determining evolutionary fitness (Szmant-Froelich 1986; Vermeij et al. 2003; Edmunds 2005; Vermeij 2006; Weil et al. 2009).

Organisms such as corals are composed of repetitive units called modules which together form a complete colonial unit. This form of construction allows clonal organisms to exhibit a variety of asexual modes of reproduction which are usually absent in aclonal organisms. Asexual reproduction produces genetically identical clones which perpetuate a genet's genotype and permits an increase in local distribution and abundance (Hunter 1993). In contrast, sexual reproduction produces new genotypes by genetic recombination, enhancing the genetic fitness for the species (Harrison 2011). Variations in sexual reproduction in corals include hermaphroditism or gonochorism. Both categories can be further subdivided into brooders and spawners.

Documented forms of asexual reproduction include intratentacular and extratentacular budding (Goreau and Goreau 1959; Richmond 1985), formation of polyp balls (Rosen and Taylor 1969), bail-out of polyps (Sammarco 1982), polyp expulsion (Kramarsky et al. 1997), gemmulation (Weil 2000), polyp fragmentation (Highsmith 1982; Lirman 2000) and asexual larvae (Stoddart 1983). Budding is a type of polyp fission which allows colony growth by increasing the number modules and gives rise to the different colonial growth forms (Goreau and Goreau 1959; Wijsman-Best 1977). Single adult polyps may exit the colony, with a fragment of skeleton, known as polyp expulsion, or naked, known as polyp bail-out and then regenerate a skeleton. Polyps may also be ejected in groups of up to 30 units known as polyp balls. Gemmae are the result of calcified ridge outgrowths which can detach and form new colonies. Fragmentation refers to the mechanical breakage of a coral typically by extrinsic mechanisms such as bioerosion, predation, and wave action (Smith and Hughes 1999). Asexual larvae have been proposed to be the product of internal parthenogenesis from gastrodermal tissues (Muir 1984).

Sexual Pattern: Hermaphroditism and Gonochorism

Hermaphroditism species produce both male and female gametes. This may occur in asimultaneous fashion, where spermaries and oocytes develop at the same time, or sequentially, where the individual will produce both gametes, but not at the same time. In simultaneous hermaphrodites, the term syngonic is used if ovaries and spermaries develop together within the same mesentery and digonic refers to eggs and spermaries which form in separate mesenteries (Policansky 1982). Thoeretically, sequential hermaphrodites may be further categorized as sequential cosexual, where one gamete will be produced followed by other within the same season or sequential alternating, where the gametes produced will alternate each season. Furthermore, if

male gametes are produced first, the term protandrous is utilized; if female gametes are produced first it is denominated protogynous (Policansky 1982). Sequential hermaphroditism has yet to be demonstrated to occur in corals although it has been documented in other cnidarians (Policansky 1982; Fautin 1990).

Hermaphroditism (n=295) is currently considered to be the dominant form of sexuality in scleractinians over gonochorism (n=109), while mixed sexuality is present only very rarely (n=12). The vast majority of studied scleractinian species (n=354) utilize broadcast spawning as the preferred developmental mode over brooding (n=61), the remainder species either reproduce through both methods (n=13) or conflicting information is available (n=16) (Fadlallah 1983; Harrison 1985; Richmond and Hunter 1990, Harrison 2011). Hermaphroditism is the dominant sexual pattern in Mussidae and is currently reported in 14 of the 15 studied species (Baird et al. 2009) (Table 1). There is documentation of hermaphroditism in *Mycetophillia* (Szmant-Froelich1986; Morales 2006), *Scolymia* (Willis et al. 1985; Pires et al. 1999), *Symphillia* (Willis et al. 1985; Babcock et al. 1986), *Lobophyllia* (Willis et al. 1985; Babcock et al. 1986; Shlesinger et al. 1998), *Mussa* (Steiner, 1993), *Mussimillia* (Pires et al. 1999), *Cynarina* (Shlesinger et al. 1998), and *Acanthastraea* (Willis et al. 1985; Babcock et al. 1986; Heyward et al. 1987; Wilson and Harrison, 1997; Shlesinger et al. 1998). Reproductive studies on the sexual patterns of *Isophyllia* are limited only to histological observations by Duerden (1902). It was concluded that only oocytes were noted in some colonies of *I. sinuosa* and thus the species has since been tentaively classified as a gonochoric mussid along with *M. ferox* (Szmant 1986). This record dates back to the turn of the previous century and must be verified. No data currently exists on the sexual pattern of *I. rigida.*

Table 1 Reproductive Summary of the traditional Mussidae

Genus	Species	Sexual Patterm	Developmental Mode	Source
Blastomussa	*merleti*			
Blastomussa	*wellsi*			
Micromussa	*minuta*			
Micromussa	*diminuta*			
Micromussa	*amakusensis*			
Acanthastrea	*subechinata*			
Acanthastrea	*lordhowensis*	H	BCS	Wilson and Harrison (1997)
Acanthastrea	*brevis*			
Acanthastrea	*echinata*	H	BCS	Willis et al. (1985); Babcock et al. (1986)
Acanthastrea	*rotundoflora*			
Acanthastrea	*hemprichii*			
Acanthastrea	*faviaformis*			
Acanthastrea	*bowerbanki*			
Acanthastrea	*maxima*			
Acanthastrea	*hillae*			
Acanthastrea	*ishigakiensis*			
Lobophyllia	*diminuta*			
Lobophyllia	*pachysepta*			
Lobophyllia	*serratus*			
Lobophyllia	*corymbosa*	H	BCS	Babcock and Heyward (1986); Babcock et al. (1986); Harriot (1993); Heyward et al. (1987)
Lobophyllia	*hemprichii*	H	BCS	Willis et al. (1985)
Lobophyllia	*dentatus*			
Lobophyllia	*hataii*			
Lobophyllia	*flabelliformis*			
Lobophyllia	*robusta*			
Symphyllia	*hassi*			
Symphyllia	*wilsoni*			
Symphyllia	*erythraea*			
Symphyllia	*recta*	H	BCS	Marshall and Stephenson (1933); Willis et al. (1985); Babcock et al (1986)
Symphyllia	*radians*	H	BCS	Babcock et al. (1986)
Symphyllia	*agaricia*			
Symphyllia	*valenciennessii*			
Scolymia	*vitiensis*	H	BCS	Willis et al. (1985)
Scolymia	*australis*			
Australomussa	*rowleyensis*			

Indophyllia	*macassarensis*			
Cynarina	*lacrymalis*			
Mussa	*angulosa*			
Scolymia	*cubensis*			
Scolymia	*lacera*			
Scolymia	*wellsi*	H	BR	Pires et al. (2002)
Mycetophyllia	*lamarckiana*	H	BR	Morales (2006)
Mycetophyllia	*ferox*	G? → H	BR	Szmant-Froelich (1984); Szmant-Froelich (1986); Morales (2006)
Mycetophyllia	*reesi*			
Mycetophyllia	*danaana*	H	BR	Morales (2006)
Mycetophyllia	*aliciae*	H	BR	Morales (2006)
Mussismillia	*hispida*	H	BCS	Pires et al. (1999); Neves and Pires (2002)
Mussismillia	*braziliensis*	H	BCS	Pires et al. (1999)
Mussismillia	*hartii*	H	BCS	Pires et al. (1999)
Isophyllia	*sinuosa*	G?	BCS	Duerden (1902)
Isophyllastrea	*rigida*			

Gametogenesis

Coral gametogenesis is the collective process by which spermatocytes and oocytes are produced through meiosis; known as oogenesis and spermatogenesis, respectively. Since scleractinian corals exhibit diploblastic tissue organization and have no dedicated reproductive organs, production of oocytes and spermatocytes is relegated to germ cells of endodermal origin (Szmant-Froelich et al. 1980). Germ cells must migrate into the mesoglea, an endodermal connective tissue found in the bodies of coelenterates, before starting to differentiate. Spermaries usually remain attached to the mesoglea as they develop; however, as oocytes become numerous and enlarge they are pushed out of the mesoglea and into the endodermal portion of the mesenteries. Szmant-Froelich et al. (1985) devised a classification system for characterizing the maturation of spermatocytes into 5 stages and oocytes into 4 stages which is broadly used in reproductive descriptions (Table 2). Planulae are also classified in 4 stages in brooding corals.

Maturation stages are differentiated based on color, shape, size and location. Coral spermaries typically exhibit a grainy appearance and stain red and maroon. Oocytes appear as large cells with a pronounced nucleus and tend to stain pink, orange and red. Lipids granules stored in the cytoplasm give oocytes a bubbly appearance particularly in later stages of development. Planulae are distinguished by their size, thick ectoderm and the presence of zooxanthellae within their structure. Early stage planulae are slightly larger than oocytes and in later stages may become large enough to take up large portions of the coelenteron.

Table 2 Gametogenetic Stages in Corals adapted from Szmant-Froelich et al. (1985)

	Oocytes	Spermatocytes	Planulae
Stage I	Enlarged interstitial cells w/ large nucleus in mesoglea	Small clusters in interstitial cells in mesoglea	Same size and staining properties as egg. Free from mesentery.
Stage II	Accumulation of small amount of cytoplasm around nuclei	Spermary boundaries distinct- small nuclei	Mesoglea present. Oral pore and coelenteron present
Stage III	Increased cytoplasm around nuclei- no vitelline membrane	Spermatocytes larger with large nuclei	Mesenteries forming as invaginations of mesoglea and endoderm
Stage IV	Oocytes full size w/ vitelline membrane. Chromatin dispersed	Spermatocytes undergoing mitosis and nuclear condensation. No tails	Well-developed septa; mature planula
Stage V	N/A	Spermatozoa with tails	N/A

Fecundity

The fecundity of clonal animals such as corals is determined by the number of gravid modules (polyps, zooids, ramets) and their reproductive output (number oocytes and planulae) (Hall and Hughes 1996) and can vary over the lifetime of the coral (Harrison and Wallace 1990). Fecundity is mainly a function of both colony size and polyp age (Kojis and Quinn 1985), although other factors are also significant. Natural factors affecting fecundity include increased depth, sedimentation and turbidity (Hogdson 1990; Kojis and Quinn 1984), elevated salinity, low tides, high sea temperatures and reduced ultraviolet light (Jokiel and York 1982; Jokiel 1985). Dislodgement (Ward 1995), fragmentation (Kojis and Quinn 1981), damage (Van Vegel and Bak 1994) and intraspecific competition (Rinkevich and Loya 1985) may result in decreased coral fecundity. Sublethal concentrations of pollution in the form of crude oil (Rinkevich and Loya 1977; Te 1991), ammonium (Cox and Ward 2002) and eutrophication of nutrients such as nitrogen and phosphorus (Tomascik and Sander 1987; Ward and Harrison 2000) also negatively affect fecundity. Bleaching (Szmant and Gassman 1990), partial colony mortality (Szmant-Froelich 1985), and disease (Weil, et al. 2009), may also result in a significant reduction or paralysis in reproductive output if the effective reproductive size of the organism falls below a particular size threshold for viable sexual reproduction.

Larval Development: Brooding & Broadcast Spawning

Planular organization is diploblastic: the ectodermal layer producing an epidermis and the endodermal layer differentiating into a mesoglea, gastrodermis, stomodaeum, coelenteron and mesenteries (Szmant-Froelich 1985). Zooxanthellae may be incorporated into the tissues through

the development and may enter through the oral pore (Chornesky and Peters 1987). Acquisition of symbionts has been linked to sexual mode: 90% of studied brooders transmit zooxanthellae directly from parent to offspring in a process known as vertical transmission. In contrast 25% of spawning species release zooxanthellae into their eggs (Baird et al. 2009).

Coral planulae may be produced by fertilization within the parent (brooding) or external fertilization within the water column (spawning) (Szmant 1986; Harrison and Wallace 1990; Harrison 2011). Spawned gametes may remain planktonic for weeks, resulting in dispersal by ocean currents over a broad area but at the cost of high mortality (Strathmann 1985). Brooders release mature planulae into the water which can settle quickly, increasing survivorship; however dispersive capabilities are reduced somewhat (Jackson 1990). The broadcast spawning modality is usually associated with large colony size, low recruitment, low adult mortality, and late sexual maturation (Van Moorsel 1983). Brooding is typically associated with small colony size, high adult mortality, early sexual maturation and longer breeding periods (Van Moorsel 1983; Ayre et al. 1997). Szmant (1986) has suggested that brooding has evolved in species which typically occupy unstable habitats where high adult mortality rates occur, and have been selected due to their high rates of successful recruitment.

Developmental mode tends to be conserved in Indo-Pacific families while multiple exceptions to the systematic trend exist within Caribbean families; possibly implicating differences in evolutionary selective pressure between the two zoogeographic regions (Szmant, 1985). Developmental mode within the Mussidae exhibit more variation in comparison to sexual pattern. Current reports indicate that 7 mussid species are gamete spawners, 2 species exhibit mixed spawning and brooding, while 4 species are documented brooders (Harrison, 2011). No data currently exists on the developmental modes for either *I. rigida* or *I. sinuosa.*

It has been proposed that broadcast spawning (Shlesinger 1998) and gonochorism (Kerr 2005) are the ancestral conditions in scleractinians, while brooding and hermaphroditism are the derived sexual characteristics. The taxonomic groupings developed by Kerr (2005) and Fukami (2008) show that 5 of 31 clades contain species with both sexual patterns, while 13 of the 31 clades contain species with both developmental modes (Baird et al. 2009). The occurrence of scattered brooding species among clades which are typically dominated by broadcast spawners implies that developmental mode is a relatively plastic reproductive characteristic compared to sexual pattern, which probably evolved independently in multiple taxa (Harrison 1985; Harrison 2011; Schlesinger 1998). It must be noted that molecular markers for many species exhibiting atypical sexual patterns have yet to be examined, making their systematic affinities uncertain (Kerr 2005; Fukami et al. 2008; Huang et al. 2009; Baird et al. 2009).

The traditional designation of scleractinian families is based on skeletal morphology and originally proposed by Vaughan and Wells (1943) and Wells (1956) is still widely in use today. However, recent molecular analyses have challenged the traditional monophyletic Mussidae concept by grouping Atlantic and Pacific members separately (Fukami et al. 2004; Fukami et al. 2008; Budd and Stolarski 2009) (Table 3). Furthermore, Atlantic Mussidae (Clade XXI) are more closely related to certain members of Faviidae (*Favia, Diploria, Manicina, Colpophyllia and Mycetophyllia)* than to their Pacific congeners (Clades XVIII-XX). Variation in sexual pattern is pretty clear within Clade XXI: 6 species are broadcast spawners and 5 species are brooders. In contrast, sexual mode is uniform: 10 species are hermaphrodites, the only exception being *I. sinuosa.*

Table 3 Summary of Reproductive Characteristics of Clades XVIII-XXI of the Taxonomy of Kerr (2005) and Fukami et al (2008).

Clade	Family	Genus	Species	Sexual Pattern	Developmental Mode	Source
XVIII	MUS	*Micromussa*	*amakusensis*			
	MUS	*Acanthastrea*	*hillae*			
XIX	MUS	*Cynarina*	*lacrymalis*			
	MUS	*Scolymia*	*sp.*			
	MUS	*Lobophyllia*	*hemprichii*	H	BCS	Willis et al. (1985)
	MUS	*Lobophyllia*	*pachysepta*			
	MUS	*Symphyllia*	*agaricia*			
	MUS	*Symphyllia*	*recta*	H	BCS	Marshall and Stephenson (1933), Willis et al. (1985), Babcock et al (1986)
	MUS	*Lobophyllia*	*corymbosa*			
	MUS	*Symphyllia*	*radians*	H	BCS	Babcock et al. (1986)
	MUS	*Scolymia*	*vitiensis*	H	BCS	Willis et al. (1985)
	PEC	*Oxypora*	*lacera*			
	PEC	*Echinophyllia*	*sp.*			
	PEC	*Echinophyllia*	*echinoporoides*			
	PEC	*Echinophyllia*	*aspera*			
	PEC	*Echinophyllia*	*orpheensis*			
XX	MUS	*Acanthastrea*	*echinata*	H	BCS	Willis et al. (1985); Babcock et al. (1986)
	MUS	*Acanthastrea*	*rotundoflora*			
XXI	FAV	*Favia*	*leptophylla*			
	MUS	*Mussismillia*	*braziliensis*	H	BCS	Pires et al. (1999)
	MUS	*Mussismillia*	*hartii*	H	BCS	Pires et al. (1999)
	MUS	*Mussismillia*	*hispida*	H	BCS	Pires et al. (1999), Neves and Pires (2002)
	FAV	*Colpophyllia*	*natans*			
	FAV	*Favia*	*fragrum*	H	BR	Szmant et al. (1985)
	FAV	*Diploria*	*labyrinthiformis*	H	BCS	Wyers (1991), Alvarado et al. (2004), Weil and Vargas (2010)
	FAV	*Manicina*	*areolata*	H	BR	Johnson (1992)
	FAV	*Diploria*	*strigosa*	H	BCS	Szmant-Froelich (1984), Wyers (1985), Soong (1991),

						Weil and Vargas (2009)
	FAV	*Diploria*	*clivosa*	H	BCS	Szmant (1986), Soong (1991), Van Veghel (1993), Weil and Vargas (2009)
	MUS	*Mussa*	*angulosa*			
	MUS	*Mycetophyllia*	*danaana*	H	BR	Morales (2006)
	MUS	*Mycetophyllia*	*aliciae*	H	BR	Morales (2006)
	MUS	*Scolymia*	*Cubensis*			
	MUS	*Isophyllia*	*Sinuosa*	G?	BR	Duerden (1902)

Characteristics of *Isophyllia* and *Isophyllastrea*

Taxonomy:

Kingdom: Animalia (Linnaeus 1758)

Phylum: Cnidaria (Hatschek 1888)

Class: Anthozoa (Ehrenberg 1834)

Order: Scleractinia (Bourne 1900)

Family: Mussidae (Ortmann 1890)

Genus: *Isophyllia* (Milne, Edwards, and Haine 1851)

Genus: *Isophyllastrea* (Matthai 1928)

Isophyllia (Milne, Edwards, and Haime 1851) and *Isophyllastrea* (Matthai 1928) are 2 closely related genera within the Mussidae (Ortmann 1890). Genera most similar to *Isophyllia* and *Isophyllastrea* include *Symphillia* and *Acanthastrea*; sometimes considered to be their Pacific counterparts. (Veron 2000) Fossil records date both genera at approximate 20 MYO in the Tethys and 5-20 MYO in the Caribbean (Veron 2000). *Isophyllastrea* was originally designated as a separate genus from *Isophyllia* by Matthai (1928) on the basis of its subcerioid growth form and

its monocentric corallites. Both genera were recently synonymized by Veron (2000) citing differences "only in minor detail". Accordingly, morphological observations by Budd and Stolarski (2009) cite variations in differentiation and the development of the secondary axes of the septal teeth as reason to uphold the original diagnosis of Matthai (1928) to separate both genera (Table 4). It remains to be determined whether the morphological and genetic differences are sufficient to indicate that *I. rigida* and *I. sinuosa* belong in separate genera or whether they should be consolidated.

Table 4 Comparison of macromorphological, micromorphological and microstructural characteristics of *I. sinuosa* and *I. rigida*. Adapted from Budd and Stolarski (2009).

Characters	***I. sinuosa***	
Macromorphological Characters		
Shape	Massive	Massive
Arrangement	Meandroid	Subcerioid
Bud Symmetry	circumoral	Circumoral
Calice/Valley Width	10-15mm	10-15mm
Center linkage	trabecular	Trabecular
Epitheca	reduced	Reduced
Calice Relief	4-10mm	4-10mm
No. of septal cycles	>3	>3
Relative costoseptal thickness	equal	Unequal
Septal Spacing per 5mm	<6	6 to 12
Minor Septa	present	Present
Confluent Costae	present	Present
Septal Lobes	absent	Absent
Micromorphological Characters		
Septal Tooth Outline	cicular	Circular
Secondary Axes	strong; axis perpendicular to septal plane	Strong
Distribution of septal tooth granulation	secondary axis	multi-directional
Septal Face Granulation	diffuse, pointed	diffuse, pointed
Area between Teeth	weakly banded	weakly banded/smooth
Columella Shape	loose threads; teeth different from septa	loose threads; teeth different from septa
Microstructural Characters		
Coenosteum	present	Present

Corralite wall	mostly parathecal; partially septothecal	mostly parathecal; partially septothecal
Calcification Centers	clusters of centers along a medial line	clusters of centers along a medial line
Structure of thickening deposits	Well-developed layers (no rings)	Layers and concentric rings

Each genus is composed of a single species: *Isophyllia sinuosa* (Ellis and Solander 1786) and *Isophyllastrea rigida* (Dana 1848), respectively. *Isophyllia multiflora* (Verrill 1901) (Figure 1E & F) was previously considered a distinct species due to a smaller corallum, narrower and closed valleys and increased number of septa/cm in comparison to *Isophyllia sinuosa*, however Cairns (1982) found differences to be within the normal range of variation of *I. sinuosa* and synonymized them. *Isophyllia erythraea* (Klunzinger 1879) was transferred to the genus *Symphillia* (Veron 2000). Both *Isophyllia* and *Isophyllastrea* are presently limited only to the greater Caribbean (Veron 2000).

Isophyllia sinuosa (Figure 1 A & C) is commonly known as sinuous cactus coral [previously *I. dipsacea* (Verrill 1901), *I. fragilis* (Verrill 1901), *I. sinuosa* (Matthai 1928)], is a small (<20cm) massive uniserial, meandroid colony with short sinuous valleys. Specimens typically exhibit 7-9 septa per cm and the 4-5 principal septa reach the columella which is trabecular and discontinuous. Septa are thin with sharp attenuated teeth. Mean valley length for colonies is 60 mm (Fenner 1993). Coloration is typically light green, lavender, or yellow with valleys and walls of contrasting colors. This coral is common in shallow protected reef habitats but has been found at depths of up to 28m (Cairns 1982; Fenner 1993; Humann 1993; Veron 2000; Budd and Stolarski 2009). Polyps are typically retracted during daytime.

Isophyllastrea rigida (Figure 1B & D) is typically known as the rough star coral [previously *Mussa rigida* (Verill 1901), *Isophyllastrea rigida* (Matthai 1928), *Isophyllia rigida* (Veron 2000)] is a massive, subcerioid colony of up to 15 cm in diameter with short, monocentric

valleys of distinctive columellae (Cairns 1982). Calices are distinct and may exhibit between 1-3 distinct centers. Single calices may have 25-30 septa and a diameter of up to 10 mm. Septae are thin, with finely pointed teeth and columellae are rudimentary, composed of loose trabeculae. Coloration is usually green, purple, or pink with contrasting valleys and walls. Species is common in spur and groove formations and is distributed between 1 to 20 m (Cairns 1982; Fenner 1993; Humann 1993; Veron 2000 Budd and Stolarksi 2009). Polyps are typically retracted during daytime.

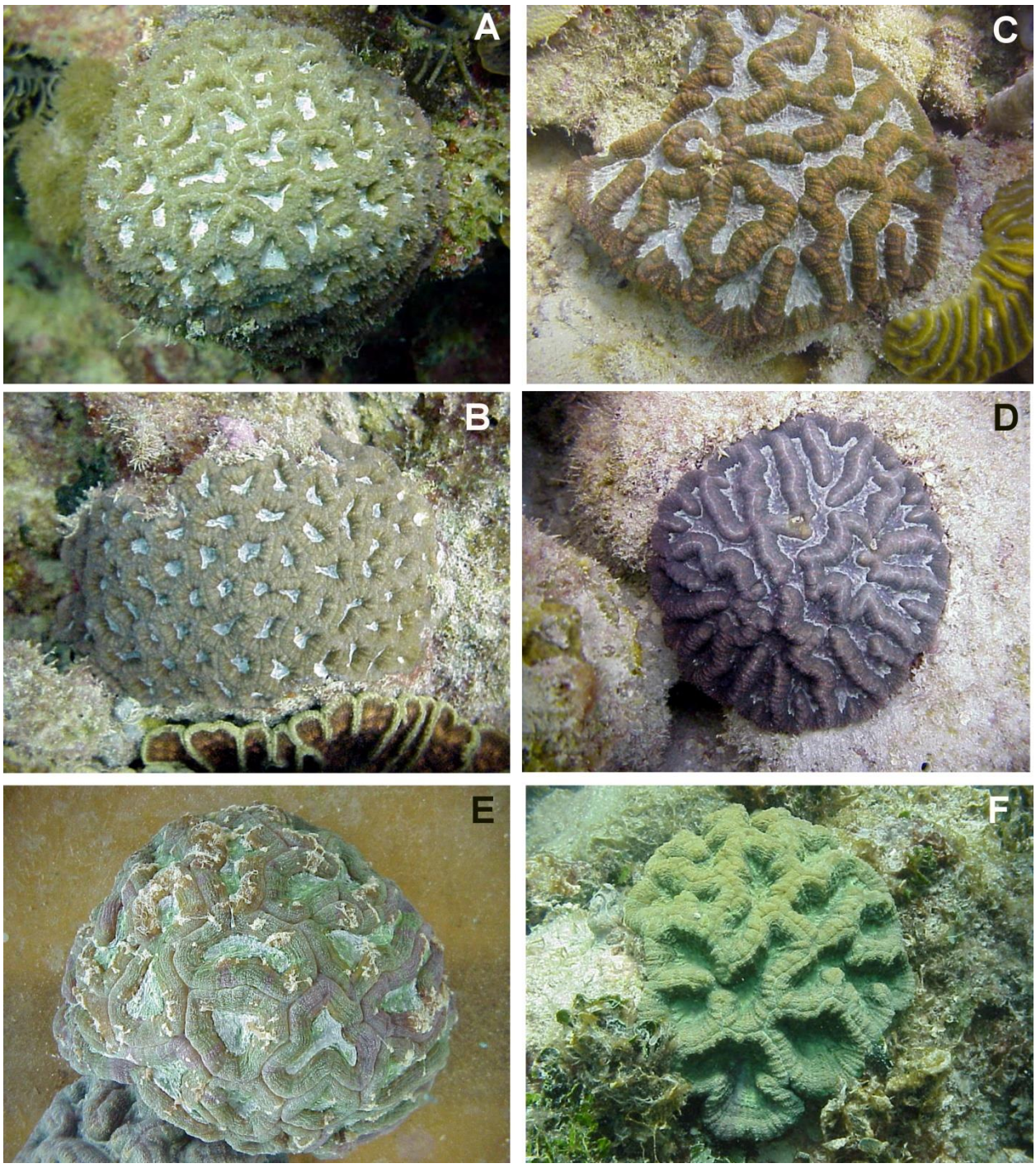


Figure 1 *Isophyllastrea rigida* (A, B) *Isophyllia sinuosa* (C, D) *Isophyllia multiflora* (E, F). Photos by Ernesto Weil.

METHODOLOGY

Main Objectives

1. To document and compare the complete sexual patterns and developmental modes for *I. sinuosa* and *I. rigida* in Puerto Rico.
2. To characterize the gametogenetic cycles over time for *I. rigida* and *I. sinuosa*, in terms of onset, phase, and duration of each cycle.
3. To characterize the distribution of gametes and larvae in the mesenteries while calculating fecundity (mesentery and polyp) utilizing histological observations.
4. To assess the variability of sexual reproduction within the genera *Isophyllia* and *Isophyllastrea* in Puerto Rico by determining differences in gametogenesis, brooding timing, fecundity.

Questions and Hypotheses

1. What is the sexual pattern and developmental mode for *I. sinuosa* and *I. rigida*?

   H_{01}: Both species are hermaphroditic.

   H_{11}: Both species are gonochoric.

   H_{02}: Both species are brooders.

   H_{12}: Both species are broadcast spawners.

   H_{03}: There are no differences in the developmental mode and sexual pattern between species.

   H_{13}: Both species show a different developmental mode and sexual pattern.

2. What is the timing of the gametogenetic cycle for *I. sinuosa* and *I. rigida*?

H_{03}: Gametogenesis occurs annually.

H_{13}: Gametogenesis occurs seasonally.

H_{04}: There are no differences in the timing of the gametogenetic cycle between species.

$H_{14:}$. Both species shows a difference in the timing of the gametogenetic cycle

3. Are there any differences in fecundity between *Isophyllia* and *Isophyllastrea* in Puerto Rico?

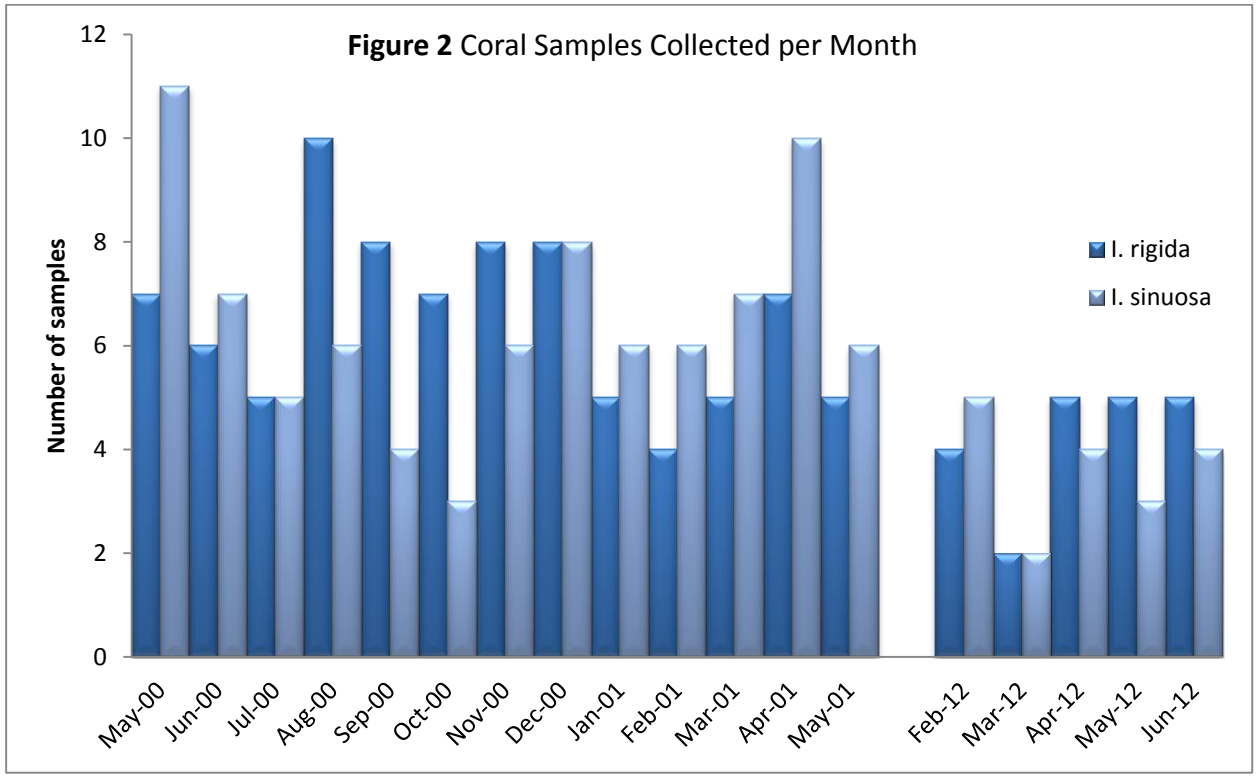
$H_{04:}$ There are no differences in fecundity between species.

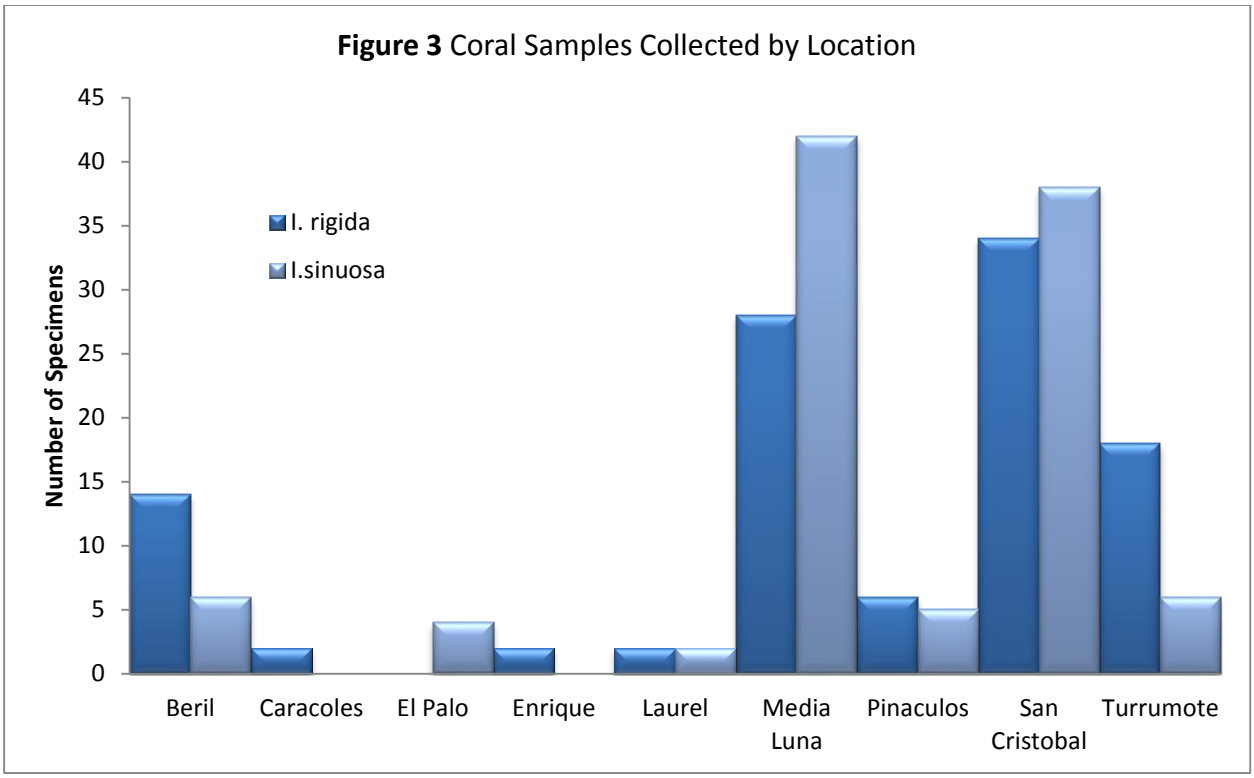
$H_{14:}$ Both species exhibit significantly different fecundity levels.

MATERIALS AND METHODS

Study Sites

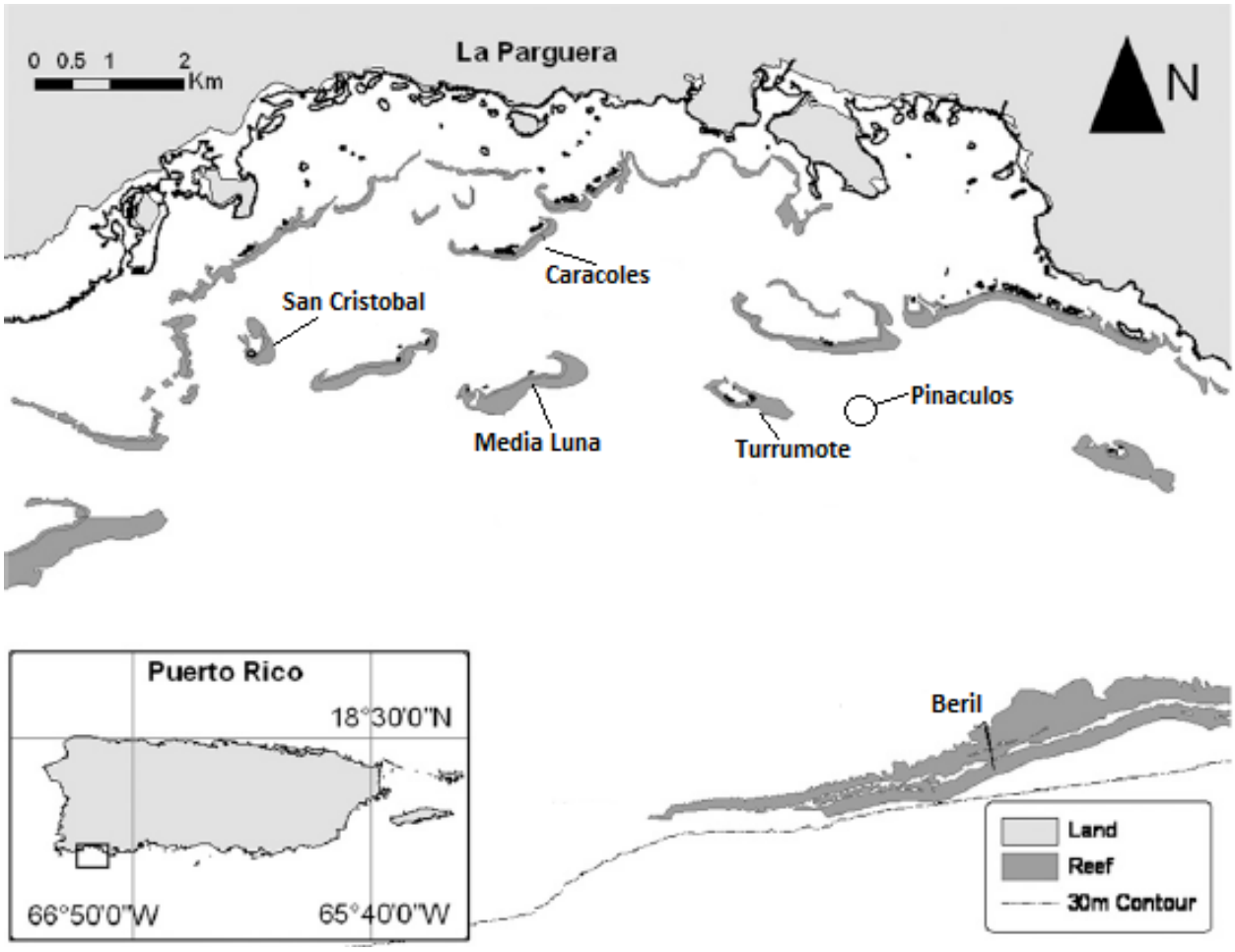
Tissue samples have been collected on a monthly basis (Figure 2) from various reef locations in La Parguera off the southwest coast of Puerto Rico (Figure 3). The following reefs were sampled: San Cristobal, Caracoles, Media Luna, Pinaculos, Turrumote and Beril. (Figure 4) Reefs were selected for specimen abundance, diving convenience and proximity to the Isla Magueyes Marine Laboratories of the Department of Marine Sciences of the University of Puerto Rico, Mayagüez Campus.





Field Collection

Between 1999 and 2001, 5 individual tissue samples were collected by Dr. Ernesto Weil every month for 14 months for 24 Caribbean coral species, including *I. sinuosa* and *I.rigida.* An additional collection of samples was made by the author during February-June 2012. Using a chisel and a hammer, one core (~2.5 cm in diameter) from the central area of each colony was removed. Cores were placed in individual, labeled plastic bags with seawater and moved to the laboratory. Tissue samples were processed and the prepared slides were used for histological analysis. Identical methods were utilized during preparation and processing of both collections.



Reef	Latitude	Longitude	Type
Beril	17°52'47.85"N	66°59'1.40"W	Bank
Caracoles	17°57'46.02"N	67° 2'8.21"W	Fringing
El Palo	17°55'56.60"N	67° 5'43.81"W	Fringing
Enrique	17°57'19.32"N	67° 2'47.69"W	Fringing
Laurel	17°56'30.47"N	67° 3'40.99"W	Patch
Media Luna	17°56'22.68"N	67° 2'43.26"W	Fringing
Pinaculos	17°56'1.13"N	67° 0'39.75"W	Patch
San Cristobal	17°55'24.88"N	67° 6'14.52"W	Fringing
Turrumote	17°56'13.56"N	67° 1'8.92"W	Fringing

Figure 4 Map of La Parguera, Puerto Rico with Study Sites. Adapted from Flynn and Weil (2009).

Laboratory Processing

Sample fixation and decalcification

Each sample core was labeled with a piece of Mylar paper and wrapped in cheesecloth. Cores were placed in a container with Zenker Formalin (Helly's solution) for 24 hours to aid in specimen preservation, and then rinsed in tap water for an additional 24 hours. Rinsed cores were placed in glass containers with 10% hydrochloric acid (HCl) solution for decalcification. HCl solutions were changed twice every day until decalcification was complete. Complete decalcification was determined by measuring residual $CaCO_3$ using the 5% ammonium oxalate test: one 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 decalcification, tissues were rinsed with distilled water and cleaned of endolithic algae, sponges and burrowing organisms that are typically found embedded in the skeleton. Tissue samples were stored in a plastic container with 70% ethanol.

Embedding and cutting

Preserved samples were sequentially dehydrated in the rotary tissue processor under incremental concentrations of ethanol (70% and 95%) and Isopropanol solution (Tissue Dry), cleaned in Xylene solution (Tissue Clear III) Samples were embedded in paraplast (Tissue Prep, melting point 56-57 ºC) using the Tissue Tec apparatus, then placed on a freezing plate (Tissue Tek) at – 3.0 ºC until the paraffin solidified. Samples were then stored in the freezer for at least 24 hours before sectioning. Using a rotary microtome (Leitz 1512) longitudinal and cross sections (7-10 μm) were obtained from the tissue samples. Strip sections were placed in a warm Boekel bath at 48 - 50 ºC, in order to allow the tissue strips stretch. The strips were lifted up on a slide dabbed

in albumin and subsequently placed on a slide warmer (Precision) at ≈ 48 ºC for about 1-2 hours. Finished tissue sample slides were then incubated at 55 ºC for at least 24 hours to allow the tissue strip to stick to glass slide and dry well for the staining procedure.

Staining

Tissue samples were stained utilizing a modified Heidenhain's Aniline-Blue method (Coolidge and Howard, 1979) to examine the maturation stages of gametocytes and embryos. Tissue samples were first deparaffinized with Xylene solution, then slowly hydrated with distilled water using sequential decreasing concentrations of ethanol solutions (100%- 95%-70%), then rinsed in deionized water for 2 minutes. Slides were stained in preheated (56 ºC) Azocarmine G solution for 15-30 minutes, then rinsed in deionized water for a few minutes. Afterward, samples were soaked in Aniline-Alcohol for 1-2 minutes, mordant in Phosphotungstic acid for 15 minutes and stained with Aniline-Blue solution for 15 minutes to differentiate cytoplasm and connective tissues. Finally, samples were sequentially dehydrated through 70%-95%-100% ethanol solutions, cleared with Xylene solution and mounted with a Cytoseal solution to seal the tissue in the slides. Tissue samples were examined with an Olympus BX 40 compound microscope couple to an Olympus DP 26 digital microscope camera. Images were captured utilizing cellSens and IrfanView software, labeled and saved onto a hard drive. Sexual pattern and the gametogenetic cycle of each species was determined by observing the gametocyte development throughout the collection year. Gametocyte maturation sequence for each species was characterized following Szmant et al. (1985) criteria.

Fecundity

Mesenterial fecundity was estimated by adding the number of eggs and larvae per mesentery.

$$fecundity_{mesentery} = \frac{\#_{eggs} + \#_{larvae}}{mesentery}$$

Polyp fecundity for each colony was estimated by counting the total number of mature eggs and larvae (Total # eggs + Total # larvae) per polyp in a colony tissue sample.

$$fecundity_{polyp} = \frac{\#_{eggs} + \#_{larvae}}{polyp}$$

RESULTS

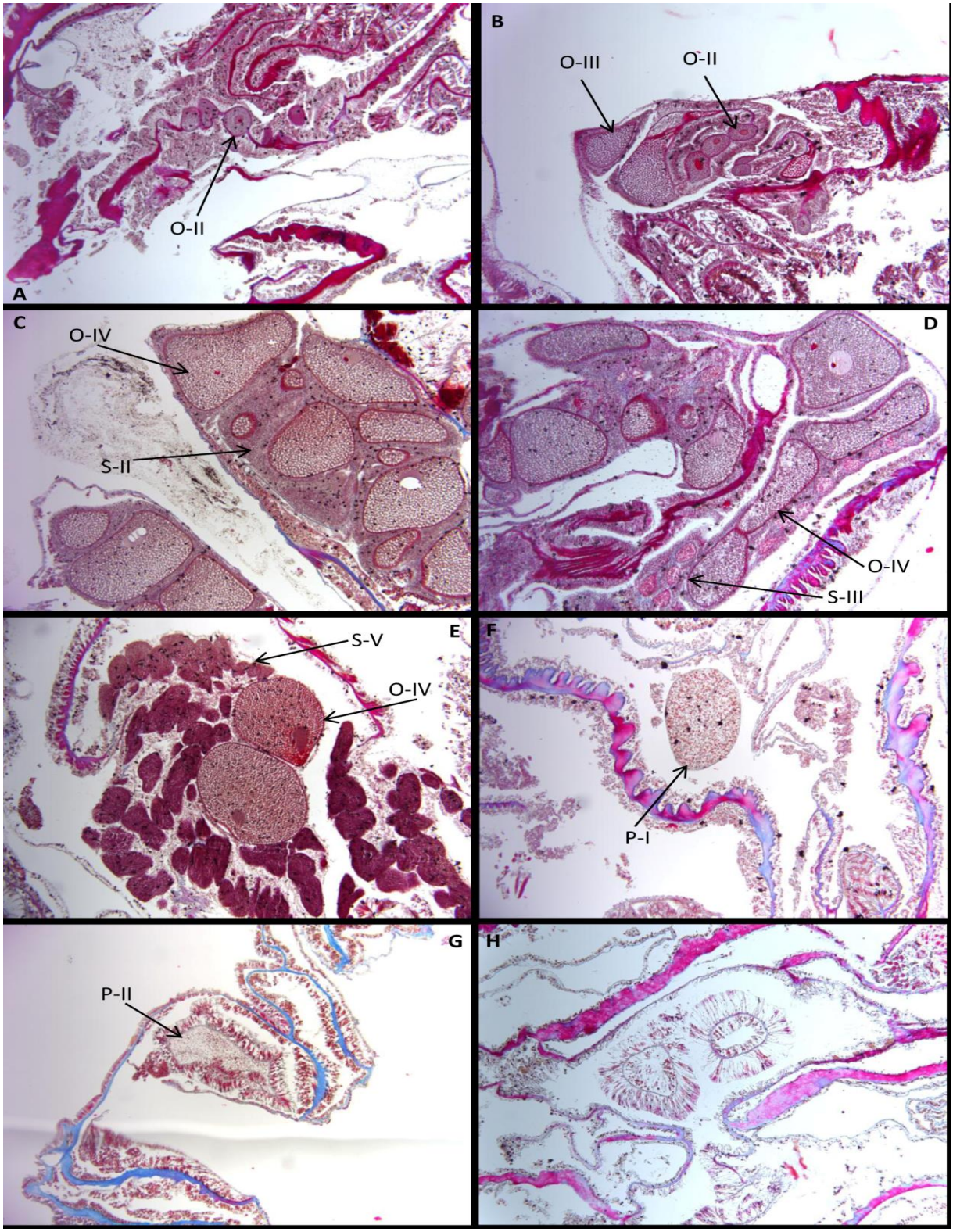
Isophyllia sinuosa

Gametogenetic cycle

I. sinuosa is a simultaneous hermaphroditic brooder. Oocytes and spermatocytes are produced within the same mesentery (dygonic) and development is consistent with an annual gametogenetic cycle (Table 5).

On histological sections (Figure 5), Stage I and II oocytes stain from a light blue to a pink, and have relatively large nuclei, with a fine grained cytoplasm. Stage III and IV are larger in appearance, stain pink to red and exhibit an abundant grainy cytoplasm. Fully mature oocytes are very large and occupy most of the space within their mesentery, their large boxy appearance making them easily distinguishable. Nuclei and nucleoli are clearly discerned throughout all stages

Oogenesis in *I. sinuosa* is an approximately 12 month process (Figure 7); new crops of eggs may appear in the wake of the previous gametogenetic cycle, often within the same polyp as late stage spermaries and/or planulae. Oogenesis begins in April-May and lasts well into March-April*;* oocytes mature for approximately 7 months prior to the appearance of spermaries; the entire oogenetic process taking between 10-12 months to complete. Young oocytes (stage I and II) appear within the linings of the mesoglea in the central regions of the polyp. Later stage oocytes detach from their mesoglea and migrate to the central regions of the mesentery where they complete their development. As the oocytes gain size and mass, space within the mesentery becomes limited and the crammed oocytes adopt more space-efficient squared shapes. Multiple crops of oocytes were often observed, and different stage eggs can often be found within the same mesentery.



F**igure 5** Developmental stages of oocytes (O) and spemaries (S) in *I. sinuosa*. **A** stage II oocytes 10X **B** Stage II and stage III oocytes 10X **C** stage II spermaries and stage IV oocytes 10X **D** stage IV oocytes and stage III spermaries10X **E** stage IV oocytes and stage V spermaries 10x **F** stage I planula **G** stage II planula 4X **H** empty mesenteries post spawn 4X.

Spermatogenesis commences approximately 7 months into the gametogenetic cycle. The first spermatocytes begin appearing in January and mature for approximately 4-5 months, until April. Spermatocytes may develop surrounding mature eggs, but may often develop in a separate mesentery. Complete development of spermaries occurs within a few months preceding spawning, fully maturing in synchrony with oocytes, although some spermaries may remain up to a month after the oocytes have been fertilized.

Early stage (I-II) spermaries stain light red or pink and form sacs with poorly defined borders joined together by thin strands of mesoglea. Stage III- IV spermaries stain red, are round and have well defined borders and remain attached by mesoglea, similar to beads on a string. Stage V spermaries stain dark red and adopt irregular shapes. Tails may be visible on spermatocytes at higher magnifications.

Planulae begin to appear within the tissues at the same time as the number of stage IV oocytes is drastically reduced. Maturation of planulae in *I. sinuosa* appears to be a rapid process as planulae were only observed within the same month as they appeared (March-April). Fertilized eggs metamorphose into gastrulae by adopting the appearance of very large oblong eggs (Stage I). They portray the same coarse grainy texture as stage IV oocytes but with few interspersed zooxanthellae and a thin cytoplasmic membrane absent cilia. Stage II planulae exhibit development of the mesoglea, an the appearance of cilia along the cell membrane, and abundant zooxanthellae within the cytoplasm. Stage III become very massive and may take up most of the space within a mesentery for themselves. Invaginations begin to appear within the cytoplasm which will mature into the planulae's mesenteries. Stage IV planulae were not observed.

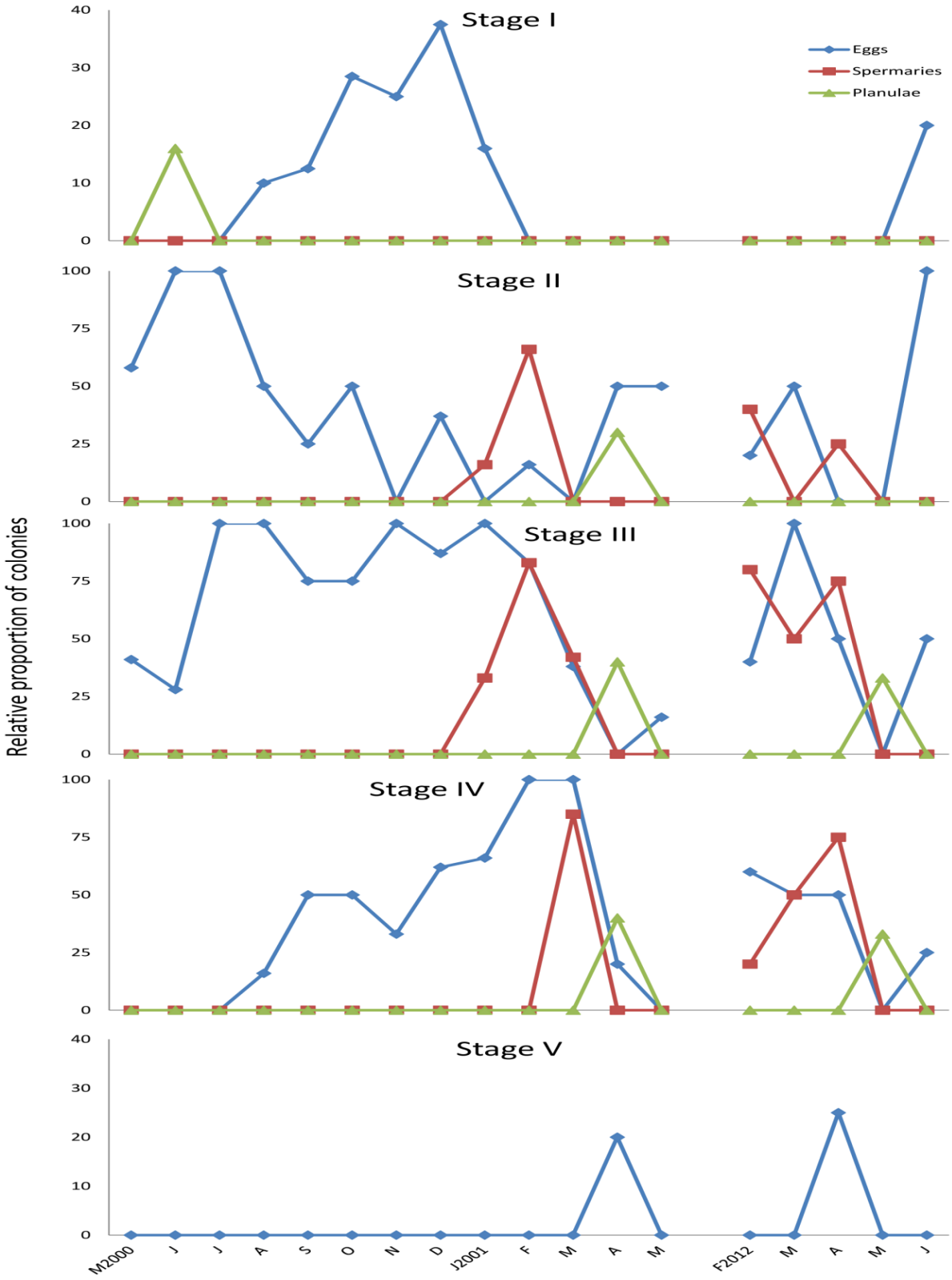


Figure 6 Adjusted values of relative proportions of colonies in each gametogenetic stage of oogenesis (I-IV), spermatogenesis (I-V) and embryogenesis (I-IV) for *I sinuosa* from May 2000 to May 2001 and February 2012 to June 2012 in La Parguera, PR.

Fecundity

Mean mesenterial fecundity values were higher in 2001 (0.87 ± 1.92) compared to mean 2012 values (0.67 ± 1.92). (Table 5) Mean number of mesenteries allocated for reproduction was also higher in 2001 (4.75 ± 3.53) compared to mean 2012 values (3.5 ± 1.71). Variability in mesenterial fecundity was high as gamete distribution was irregular; some mesenteries contained most of the eggs while many contained none at all. Mean polypal fecundity values were lower in 2001 (3.78 ± 6.42) compared to mean 2012 values (8.25 ± 2.5). No statistically significant differences in mean mesenterial fecundity (t-test, p=0.642) and polypal fecundity (t-test, p=0.22) were calculated between the years 2001 and 2012.

Table 5 Summary of fecundity values for *I. sinuosa*

	2001	2012
Fecundity/mesentery	0.87±1.92	0.68±1.35
Fecundity/polyp	3.78±6.42	8.25±2.5
Mean mesenteries used	4.75±3.53	3.5±1.73

Isophyllastrea rigida

Gametogenetic cycle

I. rigida is a simultaneous hermaphroditic brooder. Oocytes and spermatocytes are produced within the same mesentery (dygonic) and development is consistent with an annual gametogenetic cycle. (Table 5)

Oogenesis in *I. rigida* lasts approximately 11 months. The process commences in the summer months (June- August) and lasting until May-June. New crops of eggs typically appear 1 month after the release of the previous year's crop of planulae. Tissues remain mostly vacant during this month Oocytes mature for approximately 7 months prior to the appearance of

spermaries; the entire oogenetic process taking between 10-11 months to complete. Young oocytes (stage I and II) appear within the linings of the mesoglea in the central regions of the polyp. Later stage oocytes detach from their mesoglea and migrate to the central regions of the mesentery where they complete their development. As the oocytes gain size and mass, space within the mesentery becomes limited and the crammed oocytes adopt more space-efficient squared shapes. Multiple crops of oocytes were often observed, and different stage eggs can often be found within the same mesentery.

Oocytes in *I. rigida* are virtually indistinguishable from *I. sinuosa.* (Figure 6) Stage I and II oocytes stain from a light blue to a pink, and have relatively large nuclei, with a fine grained cytoplasm. Stage III and IV are larger in appearance, stain pink to red and exhibit an abundant grainy cytoplasm. Fully mature oocytes are very large and occupy most of the space within their mesentery. Nucleus and nucleolus are clearly discerned throughout all stages.

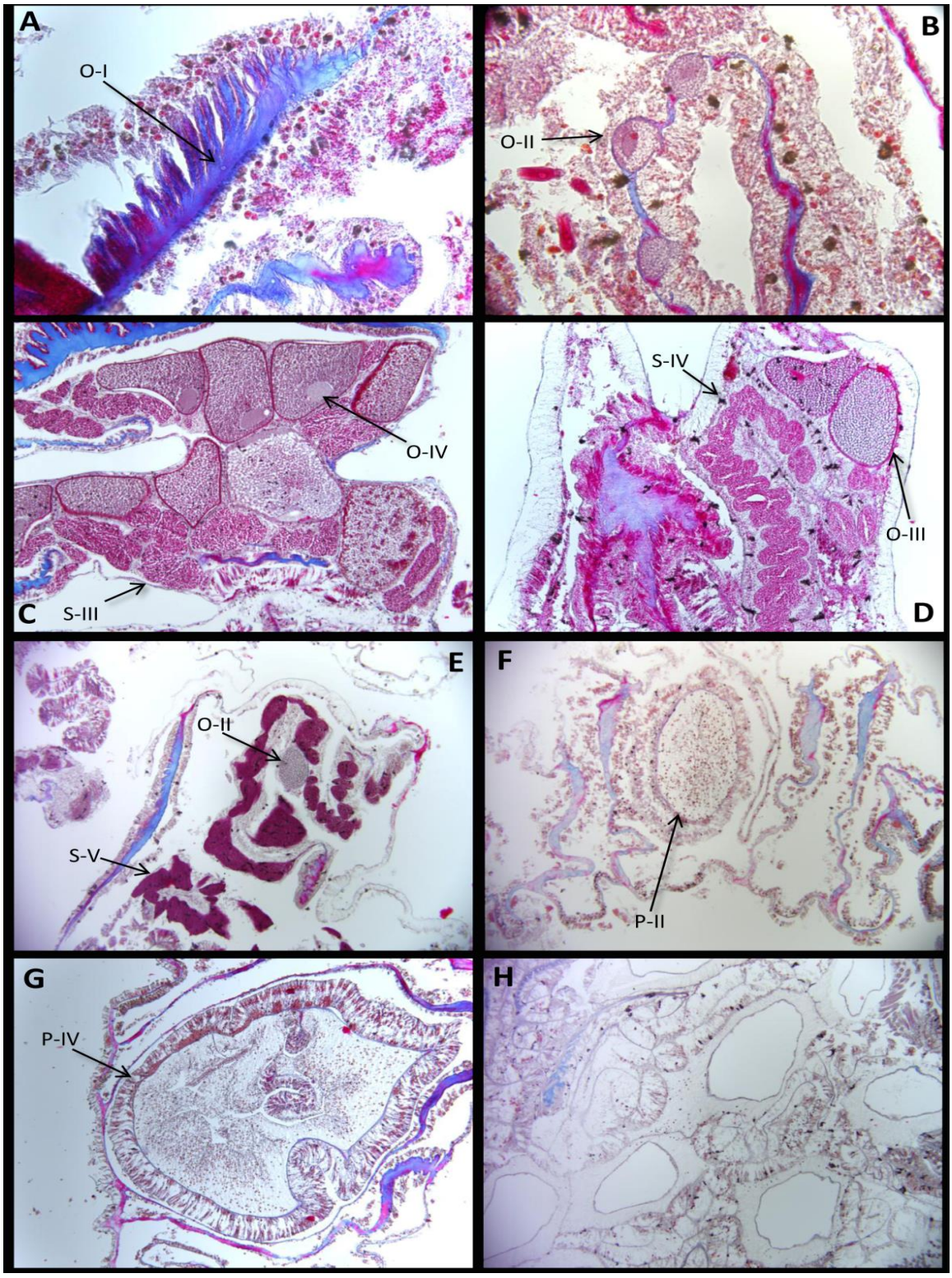
Spermatogenesis in *I. rigida* commences approximately 7 months into the gametogenetic cycle; initially appearing in April-May but sometimes as early as February. Spermatocytes mature rapidly; within 4-5 months, the whole process is finalized by June. Spermatocytes may develop surrounding mature eggs, but may often develop in a separate mesentery but maturing simultaneously with oocytes, although some spermaries may remain up to a month after the oocytes have been fertilized and released.

Early stage (I-II) spermaries stain light red or pink and form sacs with poorly defined borders joined together by thin strands of mesoglea. Stage III- IV spermaries stain red, are round and have well defined borders and remain attached by mesoglea, similar to beads on a string. Stage V spermaries stain dark red and adopt more irregular shapes. Tails may be visible on stage V spermatocytes at higher magnifications.

Gametes in *I. rigida* become fully mature during the summer (June) and planulae may be observed within the mesenteries until September. Fertilized eggs metamorphose into stage I planulae by adopting the appearance of very large oblong eggs; portraying the same coarse grainy texture as stage IV oocytes but with few interspersed zooxanthellae and a thin cytoplasmic membrane with no cilia visible. Stage II planulae begin to show signs of development of the mesoglea, and abundant zooxanthellae within the cytoplasm. Unlike in stage I, stage II planulae develop cilia on their cell membrane. Stage III planulae become very massive and may take up most of the space within a mesentery for themselves. Invaginations begin to appear within the cytoplasm which will mature into the planulae's mesenteries. Stage IV planulae have similar characteristics as stage III planulae but are so large as to take up most of the space in their mesentery.

Fecundity

Mean mesenterial fecundity values were significantly (t-test, $p=0.001$) higher for 2001 (2.76 ± 4.44) compared to mean 2012 values (0.53 ± 1.10). (Table 6) Mean number of mesenteries allocated for reproduction was also significantly (t-test, $p=0.001$) higher in 2001 (5.42 ± 1.98) compared to mean 2012 values (3.25 ± 1.89). Mean polypal fecundity were higher significantly (t-test, $p=0.001$) in 2001 (33.16 ± 4.44) compared to 2012 fecundity (7.00 ± 4.76).



F**igure 7** Developmental stages of oocytes (O) and spemaries (S) in *I. rigida*. **A** stage I oocytes in the mesoglea. **B** Stage II oocytes **C** stage III spermaries and stage IV oocytes **D** stage III oocytes and stage IV spermaries **E** stage II oocytes and stage V spermaries **F** stage II planula **G** stage IV planula **H** empty mesenteries post spawn.

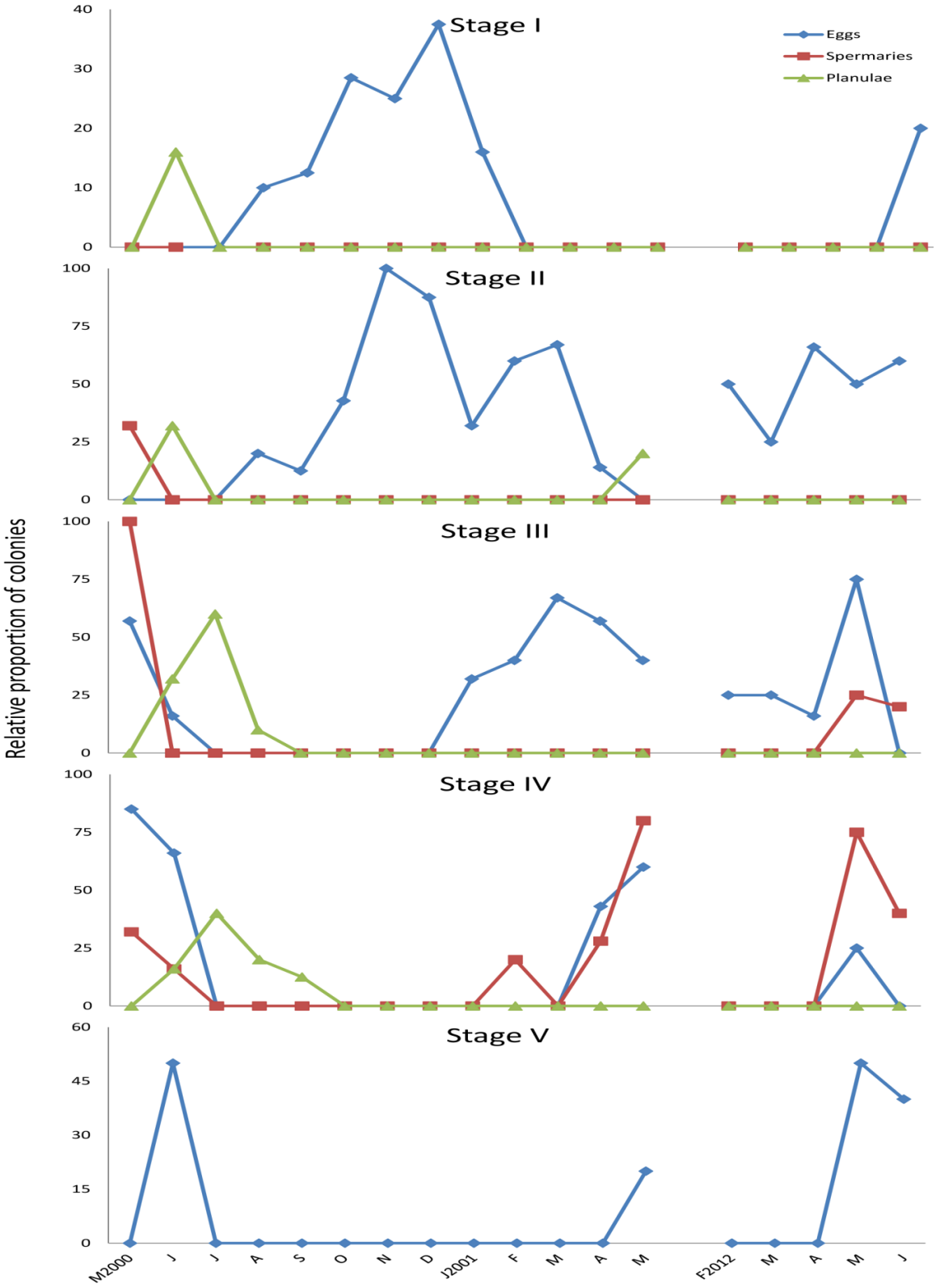


Figure 8 Adjusted values of relative proportions of colonies in each gametogenetic stage of oogenesis (I-IV), spermatogenesis (I-V) and embryogenesis (I-IV) for *I. rigida* from May 2000 to May 2001 and February 2012 to June 2012 in La Parguera, PR.

Table 6 Summary of fecundity values for *I. rigida*

	2001	2012
Fecundity/mesentery	2.76±4.44	0.58±1.10
Fecundity/polyp	33.16±4.44	7.0±4.71
Mesenteries used	5.42±1.98	3.25±1.89

The duration of the oogenetic process is similar for both species: approximately 11 months in duration in *I. rigida* and 12 months in *I. sinuosa.* In *Isophyllia*, a new crop of eggs would often overlap the previous generation spermaries (stage V) or planulae, in contrast, in *I. rigida* a month of lapse between one cycle and the next was observed.The onset of gametogenesis in *I. sinuosa* is about 1-2 months earlier than *I. rigida* and ends approximately 1-2 months sooner. In this manner, oogenesis commences in late spring in *Isophyllia* but in mid-summer in *Isophyllastrea.* In both species, oogenesis would carry on as the sole gametogenetic process for a period of 7 months and then spermatocytes would begin development for a period of approximately 5 months.

Mesenterial fecundity (eggs/mesentery) was significantly higher (T-test, p=0.046)in *I. rigida* than in *I. sinuosa* in 2001; (Figure 9A) However, differences in mesenterial fecundity were not significant for the year 2012 (P= 0.681). Consistently, polypal fecundity (eggs/polyp) was significantly (T-test, p=0.001) higher in *I. rigida* than in *I. sinuosa* in 2001; differences (Figure 9B) Significant differences were not found between both species for the year 2012 (t-test; p=0.658).

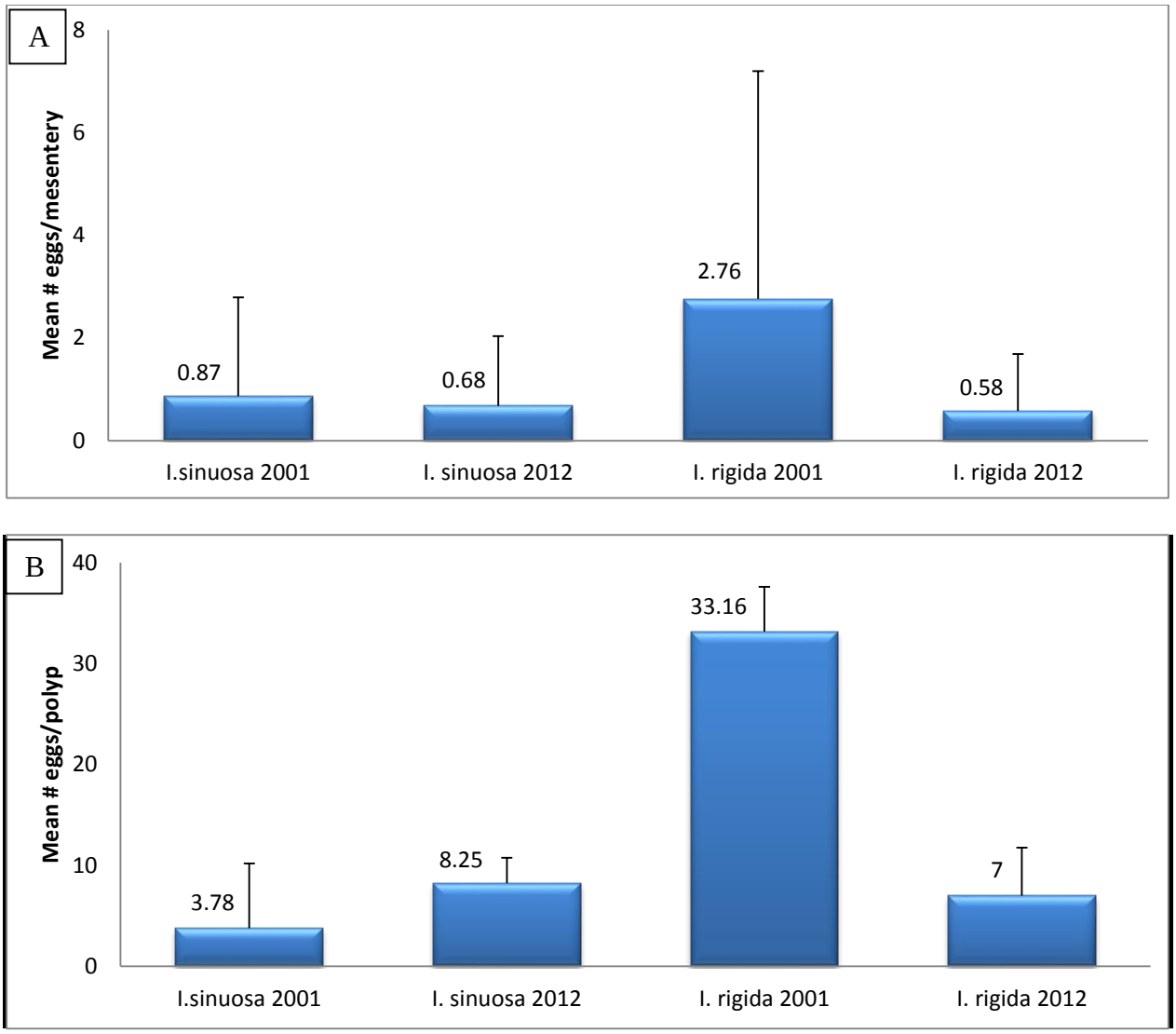


Figure 9 Comparison of sexual characteristics of *I.sinuosa* and *I. rigida.* Average mesenterial **A** (eggs/mesentery) fecundity and polyp **B** (eggs/polyp) fecundity during the years 2001 and 2012.

DISCUSSION

The recent boom in coral reproductive research underscores the importance and urgency of understanding sexual reproduction in corals. Even though other methods of reproduction may be useful as remedial measures for afflicted reefs, the long term viability of any species is directly dependent on its genetic variability and this can only arise through sexual reproduction this is effectively the keystone concept of reef preservation. Our knowledge of coral reproduction and the amount of species described to date is increasing rapidly. Prior to this study, the total amount of described Mussids numbered 16, including a mislabeled *I. sinuosa*. Including new data on *I. sinuosa* and *I. rigida* the amount of described Mussids has increased to 17. Of the Atlantic Mussids, 11 out of 14 species have already been described; *Musa angulosa, Scolymia lacera,* and *Scolymia cubensis* remain undescribed. More research is warranted as only 7 out of 37 Pacific Mussid species have been described.

Traditional morphology-based classification systems are being restructured by designating systematic affinities utilizing molecular methods in combination with morphometric analyses. The traditional Mussidae family has recently undergone extensive restructuring by separating Indopacific Mussids from their Atlantic counterparts which are more closely related to some members of the family Faviidae (Fukami et al. 2004; 2008; Budd et al 2012). The resulting 'modern' Mussidae (clade XXI) is composed of the genera *Mussa, Isophyllia, Mycetophyllia,* and Scolymia (Atlantic) under the Mussinae subfamily and the genera *Favia* (Atlantic), *Colpophyllia, Diploria, Pseudodiploria, Manicina* and *Mussismillia* under the Faviinae subfamily. Under the new classification, hermaphroditism has been exclusively documented within all the generas of the subfamily Mussinae: *Mycetophillia* (Szmant-Froelich 1986; Morales 2006), *Scolymia* (Pires et

al. 2000) and *Mussa* (Steiner 1993) and within the subfamily Faviinae: *Favia* (Soong 1991), *Colpophyllia* (Weil unpublished data), *Diploria* (Weil and Vargas 2009) *Pseudodiploria* (Weil and Vargas 2009), *Manicina* (Johnson 1992), *Mussimillia* (Pires et al. 1999). Results of this study confirm the dominant pattern of sexual reproduction described for Mussid corals (Baird 2009) and further support the conservative reproductive patterns within coral families (Harrison 2011)

Sexual Pattern and Mode of Development

I. sinuosa and *I. rigida* are both simultaneous hermaphroditic brooders which exhibit vertical symbiont transmission and an annual gametogenetic cycle (Table 7). Hermaphroditism remains the dominant sexual pattern among all corals (Baird 2009) and all Mussids universally exhibit a hermaphroditic sexuality. Sexual pattern appears to be a conservative reproductive feature particularly consistent within coral families.

Mode of development within the Mussidae is mixed. Sexual mode exhibits more plasticity than sexuality (Harrison 1985). Van Moorsel (1983) suggests that sexual mode is probably a function of habitat stability, where successful recruiters would be small, rapidly maturing species, which produce many offspring over short periods but subject to high mortality rates. Thus, the sexual modality of species occupying unstable habitats would gravitate towards brooding because it increases the chances of a successful recruitment (Smant 1986) by reducing gamete and larval mortality.

A single annual gametogenetic cycle is the dominant pattern in most broadcasting corals such as *Montastraea*, *Diploria*, *Porites*, *Acropora*, *Siderastrea* (Szmant 1986; Vargas 2002; Weil and Vargas 2009) and brooding Caribbean corals like *Porites* and *Mycetophyllia* (Szmant 1986; Soong 1993; Morales 2006). Spawning in *I. sinuosa* and *I. rigida* coincided with the longer and

warmer days of the summer months, similar to other Mussid species. Van Woesik et al. (2006) showed experimentally that coral spawning schedules correlate strongly with solar insolation levels prior to gamete release; however water temperatures are highly influential in determining actual gamete maturity. Studies with the brooding coral *Pocillopora damicornis* revealed that synchronization of larval production was lost under constant artificial new moon and full moon conditions, demonstrating that planulation is linked to nighttime irradiance (Jokiel et al. 1985). Results of this study indicate differences in the timing of spawning between both species to be between 60-90 days apart. This effectively minimizes chances for hybridization while providing the advantage of reducing wasted gametes and competition for substrate (Wilson and Harrison 1997). Even small differences in the spawning time during the same day have been considered as a potential effective reproductive barrier between different species of corals (Levitan et al. 2004). Nevertheless, differential gamete maturation in the *Isophyllia* hints at the possibility of multiple spawning events during a single gametogenetic cycle; a strategy which may increase reproductive output due to space limitations within polyps. Dual spawnings have been documented in *Acanthastrea lordhowensis* (Wilson and Harrison 1997) and cannot be discarded in these species.

Acquisition of the endosymbiont *Symbiodinium* occurs directly from parent to offspring (vertical transmission), a characteristic strongly linked to the brooding modality (Baird et al. 2009). Brooded larvae are capable of motility immediately or shortly after planulation (Fadlallah 1983) in contrast to broadcast spawned propagules, which are positively buoyant and may take between 12-72 hours to become motile (Baird et al. 2009). As such, brooded larvae are much less exposed to high levels of solar radiation which may overwhelm the photosynthetic capacities of zooxanthellae producing oxygen radicals (Tchernov et al. 2004) which may cause tissue damage and mortality (Lesser et al. 1990). In this way, species with vertical transmission of symbionts may

benefit from shorter recruitment periods than their horizontal counterparts but potentially at the cost of increased susceptibility to high temperatures associated with climate change (Yakovleva et al. 2009)

Table 7 Summary of the reproductive traits of *I.sinuosa* and *I.rigida* in La Parguera, Puerto Rico

Reproductive Traits	*I. rigida*	*I. sinuosa*
Sexual Patterns	Hermaphroditic	Hermaphroditic
Mode of Development	Brooder	Brooder
Transmission of Symbionts	Vertical	Vertical
Oogenesis onset/duration	June-July	May-June
	11 months	12 months
Spermatogenesis onset/duration	Jan-Dec	Dec-Jan
	5 months	5 months
Spawning	May-Jun	Mar-Apr
Gametogenetic cycles/year	1	1

Gametogenetic cycle

A dygonic arrangement of gametes is a fairly consistent trait within hermaphroditic families and is characteristic of the Mussidae (Harrison 1985). The arrangement of gametes within close proximity of each other, suggests that self-fertilization is a feasible alternative to sexual reproduction. This potential is necessary in order for brooders to achieve reasonable reproductive success within an unstable environment (Szmant-Froelich 1985). Significant differences in the timing of the gametogenetic cycle imply that *I. sinuosa* and *I. rigida* cannot cross-fertilize under normal circumstances.

Establishing the causal factors that regulate the timing of the gametogenetic cycle is complex and in its simplest interpretation involves considering a whole array of environmental

signals ranging from sea surface temperature (SST), day length, lunar cycles, insolation, etc (Baird 2009). Willis et al. (1985) demonstrated that a difference in spawning timing between inshore and offshore reefs at the Great Barrier Reef could only have been caused by differences in SST. As such, SST is usually considered the strongest causal link in regulating reproductive processes. This makes sense because temperature is an important regulator of physiological processes, particularly at the enzymatic level. Effectively, excessive temperatures have been shown to have a teratogenic effect; negatively impacting embryologic development in corals (Randall and Szmant 2009) and may induce significant mortality in brooded larvae.

Fecundity

The number of oocytes and subsequently planulae can be interpreted as representative of the reproductive effort of the organism. Both *I. rigida* and *I. sinuosa* allocate significant amounts of time, space and energy to their reproductive endeavors. Significantly higher amounts of eggs were produced in the year 2001 compared to 2012. Average numbers of eggs per mesentery in *I. sinuosa* (2001 (0-3) and 2012 (0-2)) and *I. rigida* (2001 (0-9) and 2012 (6-10)) were very variable but still comparable to that of other Mussids: *M. ferox* (1-1.5), *M. aliciae* (3-9), *M. lamarckiana* (1-7), *M. daanana* (2-7), *D. labyrinthiformis* (8-16), *D. clivosa* (4), *D. strigosa* (4-10) (Szmant 1986; Morales 2006; Weil and Vargas 2010). Average values of eggs per polyp in *I. sinuosa* (2001 (0-3) and 2012 (0-2)) and *I. rigida* (2001 (0-9) and 2012 (6-10)) were much smaller compared to other Mussids: *M. ferox* (1-4), *M. aliciae* (0-50), *M. lamarckiana* (0-40), *M. daanana* (0-150) (Morales 2006).

Limitations

Both *I. sinuosa* and *I. rigida* are small, uncommon species. In order to collect tissue samples from the sexually active polyps in the center of the colony, colonies were halved and the remaining half was left behind to recover. This means that any given colony could only be sampled once. In an idealized reproductive study where colonies are large and accessible, individual coral heads may be sampled multiple times. Tracking the cycle of a given group of colonies essentially removes a lot of the "noise" in the data that arises from sampling different individuals every month, even though the latter method does provide truly random sampling.

I. sinuosa and *I. rigida* are distributed throughout the greater Caribbean. This study does not take into account specimens from other geographic locations which may or may not reproduce at different times due to variations in seawater temperature or higher/lower latitudes. An idealized study would sample and compare data from multiple locations where *I. sinuosa* and *I. rigida* exist in order to provide a more accurate picture of their reproductive history.

Obtaining accurate counts of oocytes, spermaries and planulae for fecundity estimates through histology is complex. Variation may be introduced from a number of sources ranging from the selection of the correct polyp on the colony, polyp size, embedding and cutting technique and variations in counting style. A common method to aid in standardizing fecundity estimates has yet to be developed and implemented and may increase the accuracy of counts and the comparability of data across different studies.

Recommendations

Currently our knowledge on *Isophyllia* and *Isophyllastrea* is still very limited. Future studies could potentially sample other geographic areas as well as utilize longer and continuous

sampling periods. An assessment of multiple environmental conditions that correlate with the gametogenetic cycle could provide valuable insights.

Scholars still debate whether both groups are similar enough to warrant grouping as a single genus (Veron 2000) or are sufficiently different to warrant separation (Budd and Stolarski 2009). Furthermore, the existence of a potential third species within the group: *I. multiflora* and its designation as a standalone species, environmental variant or hybrid has yet to be determined. This may potentially provide a critical piece of information in the systematics of the group. If *I. multiflora is* a hybrid then *I. rigida* and *I. sinuosa* may be more closely related than previously thought, and probably should be reassigned within a single genus. If on the other hand *I. multiflora* is a distinct species, then it is a species which has yet to achieve recognition and its characteristics must be described and its sexual characteristics compared to this study. Future work determining these systematic affinities through genetic and morphologic analysis could provide these insights.

CONCLUSIONS

- *Isophyllia sinuosa* and *Isophyllastrea rigida* are simultaneous hermaphroditic species; a trait they share with other Mussids.
- *Isophyllia sinuosa* and *Isophyllastrea rigida* both brood planulae within their mesenteries. Transmission of symbionts is vertical.
- Both species exhibit a long gametogenetic cycle, lasting 11 months in *I. rigida* and 12 months in *I. sinuosa*.
- Onset of ooogenesis, spermatogenesis and embryogenesis occurs earlier in *I. sinuosa* than in *I. rigida*.
- An intermingled gamete arrangement (dygonic) was observed in both species.
- *I. sinuosa* and *I. rigida* develop gametes during most of the year and can develop larvae at the same time and within the same mesenteries as oocytes and spermatocytes.
- T-tests revealed significant differences in mesenterial and polypal fecundity between both species and between the years 2001 and 2012.

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