Sexual reproduction in the Caribbean coral genus *Isophyllia* (Scleractinia: Mussidae) in Puerto Rico

Derek Soto Corresp., [1] , **Ernesto Weil** [1]

[1] Department of Marine Science, Universidad de Puerto Rico, Recinto de Mayagüez, Mayagüez, Puerto Rico, United States

Corresponding Author: Derek Soto
Email address: derek.soto@upr.edu

The sexual pattern, reproductive mode, and timing of reproduction of *Isophyllia sinuosa* and *Isophyllia rigida,* two Caribbean Mussids, were assessed by histological analysis of specimens collected monthly during 2000-2001. 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 sequential maturation for extended planulation. Oocytes begin development 7-8 months prior to spermaries; beginning in May in *I. sinuosa* and August in *I. rigida*. Gametes of both sexes matured simultaneously; May-June in *I. rigida* and March-April in *I. sinuosa*. Planulae were observed in *I. sinuosa* during April and in *I. rigida* from June through September. Significantly higher polyp and mesenterial fecundity were found in *I. rigida* compared to *I. sinuosa*. Significantly larger oocyte sizes were found in *I. sinuosa* than in *I. rigida,* however significantly larger planula sizes were *I. rigida* compared to *I. sinuosa.* Hermaphroditism is the exclusive sexual pattern within the Mussidae; brooding has also been documented within the related Mussid genera *Mussa, Scolymia* and *Mycetophyllia*. These results represent the first description of the sexual characteristics of *I. rigida* and refute the previous description for *I. sinuosa.*

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Derek Soto · Ernesto Weil

Department of Marine Sciences, University of Puerto Rico
Call Box 9000, Mayaguez PR 00681
derek.soto@upr.edu

ABSTRACT

The sexual pattern, reproductive mode, and timing of reproduction of *Isophyllia sinuosa* and *Isophyllia rigida*, two Caribbean Mussids, were assessed by histological analysis of specimens collected monthly during 2000-2001. 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 sequential maturation for extended planulation. Oocytes begin development 7-8 months prior to spermaries; beginning in May in *I. sinuosa* and August in *I. rigida*. Gametes of both sexes matured simultaneously; May-June in *I. rigida* and March-April in *I. sinuosa*. Planulae were observed in *I. sinuosa* during April and in *I. rigida* from June through September. Significantly higher polyp and mesenterial fecundity were found in *I. rigida* compared to *I. sinuosa*. Significantly larger oocyte sizes were found in *I. sinuosa* than in *I. rigida*, however significantly larger planula sizes were *I. rigida* compared to *I. sinuosa*. Hermaphroditism is the exclusive sexual pattern within the Mussidae; brooding has also been documented within the related Mussid genera *Mussa, Scolymia* and *Mycetophyllia*. These results represent the first description of the sexual characteristics of *I. rigida* and refute the previous description for *I. sinuosa*.

Introduction

Reproduction in corals consists of a sequence of events which includes: gametogenesis, spawning (broadcasters), fertilization, embryogenesis, planulation (brooders), dispersal, settlement and recruitment (Harrison and Wallace 1990). The success of the reproductive effort is determined largely by 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 1986; Edmunds 2005; Vermeij 2006; Weil et al. 2009b; Pinzon and Weil 2011) which is defined as the product of sexual output (fecundity) and survivorship (Meitz et al. 1992). Consequently, the ability of coral species to adapt to modern-day environmental pressures depends greatly on the ability of species to reproduce effectively.

The reproductive characteristics of some scleractinian groups have been more thoroughly studied than others, however, little is known about the reproductive patterns of many Caribbean coral species and some of the available information is conflictive or incomplete (Fadlallah 1983; Harrison, 1990; 2011; Weil and Vargas 2010; Pinzon and Weil, 2011). Of the approximately 60 Caribbean zooxanthellate coral species reported, thorough descriptions of their reproductive characteristics and cycles are available for 19 species; many other studies available provide partial or conflicting results (Weil 2003; Weil and Vargas 2010; Harrison 2011). Reproductive studies of the sexual patterns of *I. sinuosa* were among the first studies of such nature performed in the Caribbean (Duerden 1902). These were limited to histological observations of oocytes in a few colonies of *I. sinuosa* (Fig. 1A, B) and the species is classified as gonochoric. This characterization contrasts with the reproductive mode of other studied Mussids which are classified as hermaphroditic. Currently, there is no information available on the reproductive biology for *I. rigida* (Fig. 1C, D).

This study characterizes the reproductive biology of *I. rigida* and *I. sinuosa* in terms of sexual pattern, mode of development, gametogenetic cycles, and fecundity. These fundamental

aspects of the physiology of this taxa are understudied. Knowledge of the reproductive biology and ecology of coral species is important for the interpretation of their population and ecological dynamics, their patterns/potential for dispersal, and their local and geographical distribution. The threats currently faced by coral reefs and the ongoing global effort to understand why corals are dying highlight the need to expand our understanding of basic coral physiology.

Materials and Methods

Sampling for this study was carried out at La Parguera Natural Reserve, off the southwest coast of Puerto Rico (Fig. 2). This complex reef environment is among the many regions experiencing deterioration by anthropogenic and environmental climate influences at local and global scales. Coral reefs in La Parguera are important local economic drivers, supporting artisanal and recreational fishing, tourism, recreational activities and also protect coastal settlements, seagrass communities and other wetland habitats from the effects of hurricanes and coastal erosion (Ballantine et al. 2008).

At least five tissue samples from different colonies were collected monthly for 14 months between March 2000 and May 2001 (Fig. 3A). A total of 89 samples of each species were collected. Samples were collected from San Cristobal reef (17°55'24.88"N 67° 6'14.52"W), Caracoles reef (17°57'46.02"N, 67° 2'8.21"W), Media Luna reef (17°56'22.68"N, 67° 2'43.26"W), Pinaculos (17°56'1.13"N, 67° 0'39.75"W), Turrumote reef (17°56'13.56"N, 67° 1'8.92"W) and Beril (17°52'47.85"N, 66°59'1.40"W) (Fig. 3B, Fig. 4). Sampling locations were selected for specimen abundance, diving logistics and proximity to the Department of Marine Sciences, University of Puerto Rico.

Samples cores (~2.5 cm in diameter) were collected using a chisel and a hammer. One core or fragment from the central area of at least 5 colonies of each species was removed. Cores

were placed in individual, labeled plastic bags with seawater and transported to the laboratory for processing. Each sample core was labeled with a piece of Mylar paper and wrapped in cheesecloth to protect the tissues during handling. Cores were placed in a container with Zenker Formalin (Helly's solution) for 24 hours, and then rinsed in tap water for an additional 24 hours. Rinsed cores were placed in glass containers with 10% HCl solution for decalcification. HCl solutions were changed twice every day until decalcification was complete. Decalcification was determined by measuring residual $CaCO_3$ using the 5% ammonium oxalate test: 1 ml of 5% ammonium oxalate was added to 5 ml of the HCl solution in the containers and allowed to stand for five minutes; a white precipitate indicates an incomplete decalcification while a clear solution indicates complete decalcification.

After decalcification, tissues were removed from the cheesecloth, rinsed with distilled water and cleaned of endolithic algae, sponges and burrowing organisms. Tissue samples were stored in plastic tissue holders in 70% ethanol until ready for embedding. Preserved samples were sequentially dehydrated in the rotary tissue processor under incremental concentrations of ethanol (70% and 95%), isopropanol solution (Tissue Dry), cleaned in xylene solution (Tissue Clear III) Samples were embedded into Paraplast blocks (Tissue Prep, melting point 56-57 ºC) using a Tissue Tek Rotary Tissue Processor, 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) 8-10 strip sections were obtained from each embedded block. Strip sections were placed in a warm Boekel bath at 48-50 ºC, in order to allow the tissue strips to stretch. The strips were lifted up on a slide and placed on a slide warmer (Precision) at ≈ 48 ºC for about 1-2 hours. Finished tissue sample slides were then incubated at room temperature for at least 24 hours to allow the tissue strip to dry and adhere to the glass slide.

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 15-20 minutes, then rinsed in deionized water for a few minutes, soaked in aniline-alcohol for 8 minutes, mordant in phosphotungstic acid for 15 minutes and stained with aniline-blue solution for 15 minutes to differentiate cytoplasm and connective tissues. Samples were then sequentially dehydrated through 70%-95%-100% ethanol solutions, cleared with xylene solution and preserved with slide covers and Cytoseal glue.

All thin slides of tissue samples were examined under an Olympus BX40 compound microscope coupled to an Olympus DP26 digital microscope camera. Images were captured utilizing Olympus cellSens 1.7 imaging software. The sexual pattern, gametogenetic cycle and fecundity of each species were determined by observing the gametocyte development throughout the collection year. Gamete stages were characterized according to Szmant-Froelich et al. (1985). Oocyte sizes were obtained using cellSens, by taking perpendicular measurements at the cells widest point. Cell length and width measurments were used to calculate geometric area. Cell length and width measurements were used to calculate geometric area. Fecundity was assessed by counting oocytes per mesentery (*I. sinuosa* n=120; *I. rigida* n=60) and per polyp (*I. sinuosa* n=10; *I. rigida* n=5) on histologic cross-sections during during months with the highest proportion of mature oocytes (*I. sinuosa* April 2001 n=5; *I. rigida* May 2001 n=5).

In April 2012, several presumed gravid colonies of each species were collected and placed in an open seawater aquarium system to observe planulation. Two colonies of each species were placed within 6 gallon aerated aquariums under continuously circulating seawater and daylight synchronized lights. Specimens were placed under mesh-lined PVC pipes allowing water to

freely circulate. Colonies were left undisturbed overnight and traps were checked daily for larvae over a 90-day period.

Statistical Analyses

Results are expressed as means ± standard error. All statistical tests were performed using the RStudio 0.99.484 software platform (R Studio Team, 2015) using the stats package (R Core Team, 2015). Normality was assessed using the Shapiro-Wilk test performed with the R function shapiro.test. Equality of variance was tested using the F test performed with the R function var.test. Differences in fecundity were tested by means of a Wilcoxon rank sum test with continuity correction performed with the R function wilcoxon.test.

Collection Permit

All coral tissue samples were collected under a General Collection Permit granted by the Puerto Rico Department of Natural Resources (DNER) to the Faculty of the Department of Marine Sciences UPRM.

Results

Results of microscopic observations indicate that both *I. sinuosa* and *I. rigida* are simultaneous hermaphroditic (gametes of both sexes are present in a single individual at the same time) dygonic (gametes of both sexes are produced within the same mesentery) brooders (bear live young) characterized by a single annual gametogenic cycle. Although spawning was not directly observed for these species, histological data suggests that both species spawn in the spring; *I. sinuosa* reached maturity in April and early May while *I. rigida* matured in June.

I. sinuosa

Stage I oocytes are small (78.92±13.15 μm^2), stain pink and are characterized by sparse cytoplasm and prominent nuclei (Fig. 4A). Oocytes originate within the linings of the mesoglea

in the central regions of the mesenteries. Stage II oocytes are larger than stage I cells (144.54±43.19 μm^2), exhibit prominent nuclei and abundant cytoplasm (Fig. 4B). Stage III oocytes are larger than stage II (264.51±37.24 μm^2), tend to have a round shape, stain pink or red, and are characterized by many cytoplasmic globules which produce a grainy appearance (Fig. 4B). Stage IV oocytes are larger and boxier than stage III (376.69±73.20 μm^2). This stage is characterized by dark staining nuclei and large globules in the cytoplasm (Fig. 4C, D & E).

No stage I spermaries were found, suggesting this stage occurs briefly and/or is difficult to differentiate using the current method. Stage II spermaries form small poorly defined bundles which form in the mesenteries surrounding oocytes (Fig. 4D). Stage III spermaries form small sacs with well-defined borders (Fig. 4C) and contain bright red staining spermatids. Stage IV spermaries stain dark red and are larger than stage III (Fig. 4E). Tails visible on spermatozoa at high magnification are indicative of stage V spermaries. Spermary sizes were not measured.

Stage I planulae are approximately the same size as stage IV oocytes (404.07 μm^2) and stain pink. During this stage, zooxanthellae become visible within the planulae, confirming vertical symbiont transmission. Stage II planulae (455.45±32.84 μm^2) are characterized by an outer layer composed of columnar cells which contain nematocysts and cilia (Fig. 4F). Developing mesenteries can be seen developing within the gastrodermis of stage III planula (501.98±44.68 μm^2). Stage IV planula were not observed.

The gametogenic cycle of *I. sinuosa* is summarized in Fig. 5. Weekly sea surface temperature measurements for La Parguera are included for reference (Fig 5A). Oogenesis in *I. sinuosa* lasts approximately 11 months (Fig 5B). Onset of oogenesis was determined to occur during May 2000 and during April 2001. Onset of oogenesis was determined as the month of appearance of stage I and II oocytes after the culmination of the previous gametogenic cycle. Stage II oocytes were prevalent in tissues during all months sampled except during November

2000 and January 2001. Stage III oocytes were observed in all sampled months except April 2001. Stage IV oocytes were observed between August 2000 through May 2001.

Spermatogenesis takes places during 4 months (Fig. 5C). Onset of spermatogenesis was not determined because stage I spermaries were not identified. Stage II spermaries were observed during January through February 2001. Stage III spermaries were visible from January through March 2001. Stage IV spermaries were present in March 2001. Stage V spermaries were present in tissues in April 2001.

Stage I-III planulae were observed in histologic sections during April 2001 (Fig. 5D). This suggests that fertilization occurred during early April (most recent Full Moon: April 9). Planulae remained visible within tissue sections briefly: only during April, suggesting planulation occurred later during that month. The appearance of planulae in tissues coincided with an increase in water temperature, suggesting seasonal synchrony in the reproductive cycle. The identification of planulae on tissue sections coincided with a sharp decrease in the proportion of colonies containing mature (IV) oocytes. No larvae were collected from specimens placed in aquaria for observation.

I. rigida

Stage I oocytes are very small (72.97±15.75 μm^2) and are characterized by sparse cytoplasm and a large nucleus (Fig. 6A). Stage II oocytes are larger than stage I cells (101.25±23.09 μm^2), are ovoid shaped and feature a prominent nucleus and nucleolus (Fig. 6A). A pink-staining nucleus and red nucleolus can clearly be identified in many stage III oocytes (148.77±49.35 μm^2) (Fig 6B). Stage IV oocytes are large (190.40±45.18 μm^2), irregularly shaped and contain large vacuoles in the ooplasma which give it a grainy appearance (Figs. 6C & D).

Stage I spermaries were not detected in *I. rigida*. Stage II were observed forming adjacent to stage III eggs (Fig. 5B). Spermaries typically adopt a spherical shape and often form in series

192 resembling a string of beads (Figs. 5B & C). Stage III spermaries form small oblong sacs and
193 stain red (Fig. 5C). Stage IV spermaries are densely packed with sperm, have irregular shapes,
194 stain dark red to brown. Stage V spermaries stain darker than stage IV (Fig. 5E) but are
195 characterized by tails on spermatozoa under high magnification. No measurements were collected
196 for spermaries.

197 Stage I planulae are approximately the same size as stage IV oocytes (approximately
198 324.01±71.64 μm^2), stain pink, and contain zooxanthellae in the epidermis. Observation of
199 zooxanthellae within planulae confirms vertical transmission of endosymbionts. Stage II planulae
200 are larger (521.27±84.18 μm^2) (Fig. 5F) and exhibit an epidermis consisting of columnar
201 epithelium similar to *I. sinuosa*. Stage III and stage IV larvae measure 818.91±82.96 μm^2 and
202 951.78±176.36 μm^2 respectively, and show clear development of the mesenteries.

203 The gametogenic cycle of *I. rigida* is summarized in Fig. 7. Weekly sea surface
204 temperature measurements for La Parguera are included for reference (Fig 7A). Oogenesis in *I.*
205 *rigida* lasts approximately 10 months. Oogenesis began during August 2000. Stage II oocytes
206 were observed in tissues in March 2000 and August 2000 to April 2000. Stage III oocytes were
207 observed in March 2000, May and June 2000 and from January 2001 through May 2001. Stage
208 IV oocytes were observed in samples collected during April through June 2000, February 2001
209 and April through May 2001.

210 Spermatogenesis in *I. rigida* is estimated to last approximately 2-3 months (Fig. 7B).
211 Onset of spermatogenesis was not determined because stage I spermaries were not identified.
212 Stage II spermaries were observed in May 2000. Stage III spermaries were visible in May 2000.
213 Stage IV spermaries were observed first in June 2000. Stage V spermaries were observed in May
214 2000.

Stage I planulae were observed in June 2000 indicating the onset of embryogenesis (Fig. 7C) which suggests a fertilization date in late May (most recent Full Moon: May 6, 2001). A late May fertilization date is supported by identification of Stage II planula during May 2001 (most recent Full Moon: May 18, 2000). Both dates coincide with an increase in local SST suggesting seasonal synchronization of the gametogenic cycle. The appearance of planulae coincided with a sharp decrease in the proportion of colonies containing mature oocytes. Stage II planulae were observed only during June 2000 and May 2001. Stage III planulae were observed from June through August 2000. Stage IV planulae were observed in tissues from June throughout September 2000. The presence of planulae in tissues collected during May through September 2000 suggests a long maturation time for this species. No larvae were collected from specimens placed in aquaria for observation.

Fecundity

Mesenterial fecundity in *I. sinuosa* was significantly higher (Wilcoxon-rank sum test, W=1208.5, $p<2.2x10^{-16}$) (11.13±0.90 oocytes/mesentery) than in *I. rigida* (1.70±0.30 oocytes/mesentery) (Fig. 8A). Polyp fecundity in *I. sinuosa* (14.55±6.44 oocytes/polyp) was significantly higher (Wilcoxon-rank sum test, W=17, p=0.014) compared to *I. rigida* (7.0±5.88 oocytes/polyp) (Fig. 8B).

Oocyte Size

Measurements of oocyte geometric area in *I. sinuosa* (range 43.94-463.79 μm^2) show an increase in the size of oocytes as maturity progresses from April through March (Fig. 9A). Mean geometric area is lowest during the month of June 2000 (97.22±28.85 μm^2) and greatest during February 2001 (333.95±74.32 μm^2). The appearance of planulae in histological sections during the month of April 2001 (459.07±45.83 μm^2)(range: 404.07-548.49 μm^2) coincides with a sharp decrease in mean geometric area of oocytes compared to the previous month (285.68±96.46 μm^2

vs. 143.28±84.07 μm^2). Measurements of oocyte geometric area in *I. rigida* (range 43.31-307.35 μm^2) also show a trend of increasing oocyte size as maturity progresses from August through June (Fig. 9B). Mean geometric area is lowest during the month of September 2000 (68.35±17.04 μm^2) and greatest during June 2000 (210.54±42.90 μm^2). Mean planulae area was greatest during the month of July 2000 (909.48±250.56 μm^2) and ranged from 241.66-1183.96 μm^2. Mean oocyte geometric area was greater in *I. sinuosa* than in *I. rigida* (Wilcoxon-rank sum test, W=43911, $p<2.13x10^{-13}$), however mean planulae geometric area was significantly higher in *I. rigida* compared to *I. sinuosa* (Wilcoxon-rank sum test, W=186, p=0.008).

Discussion

Traditional morphology-based classifications are being restructured by designating systematic affinities using 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 *Favia* (Atlantic), *Colpophyllia*, *Diploria, Pseudodiploria, Manicina* and *Mussismillia* under the Faviinae subfamily. Under the new classification, hermaphroditism has been exclusively documented within all the genera of the subfamily Mussinae: *Mycetophillia* (Szmant-Froelich 1986; Morales 2006), *Scolymia* (Pires et al. 2000; Weil unpublished data) 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) (Table 1). Results of this study confirm the dominant pattern of sexual reproduction described for

Mussid corals (Baird 2009) and provide further support for conserved reproductive patterns within coral families (Harrison 2011).

Results of this study contradict observations by Duerden (1902) that label *I. sinuosa* as a gonochoric species. This misconception has resulted in the classification of *I. sinuosa* as the sole gonochoric outlier within the traditional Mussidae, which was otherwise uniformly hermaphroditic (Duerden 1902; Fadlallah 1983; Richmond and Hunter 1990). Mode of development within the modern Mussidae is mixed; both brooding and spawning species are present. Brooding has been documented within *Mycetophyllia* (Morales 2009), *Scolymia* (Pires et al. 2000; Weil unpublished data), *Manicina* (Johnson 1992). Broadcast spawning is found in *Colpophyllia* (Weil unpublished data), *Diploria* (Weil and Vargas 2009), *Pseudodiploria* (Weil and Vargas 2009), and *Favia* (Soong 1991). Sexual mode exhibits more plasticity than sexuality (Van Moorsel 1983; Harrison 1985): contrasting modes of development existing within families and even within genera (Harrison 2011). Szmant (1986) suggested that sexual mode is potentially 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 by reducing gamete and larval mortality even in low population densities. This may partially explain why, in recent decades, brooding corals have begun to dominate some Caribbean reefs following degradation from natural and anthropogenic disturbances (Hughes 1994; Mumby 1999; Knowlton 2001; Irizarry and Weil 2009).

A single annual gametogenetic cycle is the dominant pattern in most broadcasting corals such as *Orbicella*, *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; Vermeij et al. 2004; Morales 2006). Various environmental factors

have been shown to correlate with coral reproductive cycles and may play a role in their synchronization, including sea temperature, salinity, day length, light/dark cycles and tidal cycles (reviewed in Harrison and Wallace (1990). Van Woesik et al. (2006) showed experimentally that some coral spawning schedules correlate strongly with solar insolation levels prior to gamete release; however, water temperatures are highly influential in determining actual gamete maturity. Van Woesik (2009) also demonstrated a positive correlation between the duration of regional wind calm periods and the coupling of mass coral spawnings. 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 in some species is linked to nighttime irradiance (Jokiel et al. 1985).

Long oocyte generation times, differential gamete maturation, and long brood retention times in *Isophyllia* suggest the possibility of multiple brooding events during a single gametogenetic cycle; a strategy which may increase reproductive output due to space limitations within polyps. Multiple spawning events have been documented in *Acanthastrea lordhowensis* (Wilson and Harrison 1997) and cannot be discarded in these species.

Generally, self-fertilization is not a favored method of fertilization in corals due to possibility of inbreeding depression (Knowlton et al. 1993). However, selfing is thought to be advantageous in certain sessile hermaphrodites which are ecologically distant from other mates and may have limited access to gametes of the other sex, providing a viable alternative for successful fertilization (Sawada et al 2014). The close proximity of oocytes and spermaries within the same mesentery (dygonism) in both *I. sinuosa* and *I. rigida* suggests that it is possible that self-fertilization can occur in these species. Selfing has been documented in other brooding corals such as *Seriatopora hystrix* (Sherman 2008), *Favia fragum* and *Porites astreoides* (Brazeau et al. 1998).

311 Acquisition of the endosymbiont *Symbiodinium* occurs directly from parent to offspring
312 (vertical transmission), a characteristic strongly linked to the brooding modality (Baird 2009).
313 Brooded larvae are capable of motility immediately or shortly after planulation (Fadlallah 1983)
314 in contrast to broadcast spawned propagules, which are positively buoyant and may take between
315 12-72 hours to become motile (Baird et al. 2009). As such, brooded larvae are much less exposed
316 to high levels of solar radiation which may overwhelm the photosynthetic capacities of
317 zooxanthellae producing oxygen radicals (Tchernov et al. 2004) which may cause tissue damage
318 and mortality (Lesser et al. 1990). In this way, species with vertical transmission of symbionts
319 may benefit from shorter recruitment periods than their horizontally transmitted counterparts but
320 potentially at the cost of increased susceptibility to high temperatures associated with climate
321 change (Yakovleva et al. 2009).

322 There is increasing evidence that sexual reproduction in corals is highly susceptible to
323 natural and anthropogenic stressors that reduce fecundity, fertilization success, and larval survival
324 (Harrison and Wallace 1990; Harrison 2011). Increases in sea surface temperatures as a
325 consequence of global warming have produced widespread coral bleaching events and disease
326 outbreaks with massive mortality of susceptible individuals. This worldwide decline of coral
327 reefs underscores the need for understanding sexual reproduction in corals as the only mechanism
328 capable of safeguarding their future. Sexual recombination is an important prerequisite for the
329 selection of individuals which are to be able to adapt to the pressures of a changing environment.
330 A greater understanding of the mechanisms and variables in sexual reproduction in corals, in
331 combination with knowledge of the taxonomy and variability of the species, is essential for any
332 coral reef management strategy (Harrison and Wallace, 1990).

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467 **Figures**

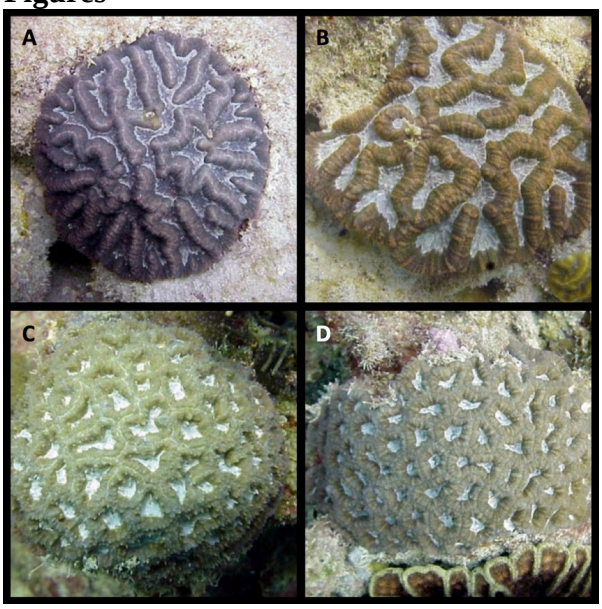

Fig. 1 (**A & B)** *Isophyllia rigida* (**C & D)** *Isophyllia sinuosa*. Photos by Ernesto Weil.

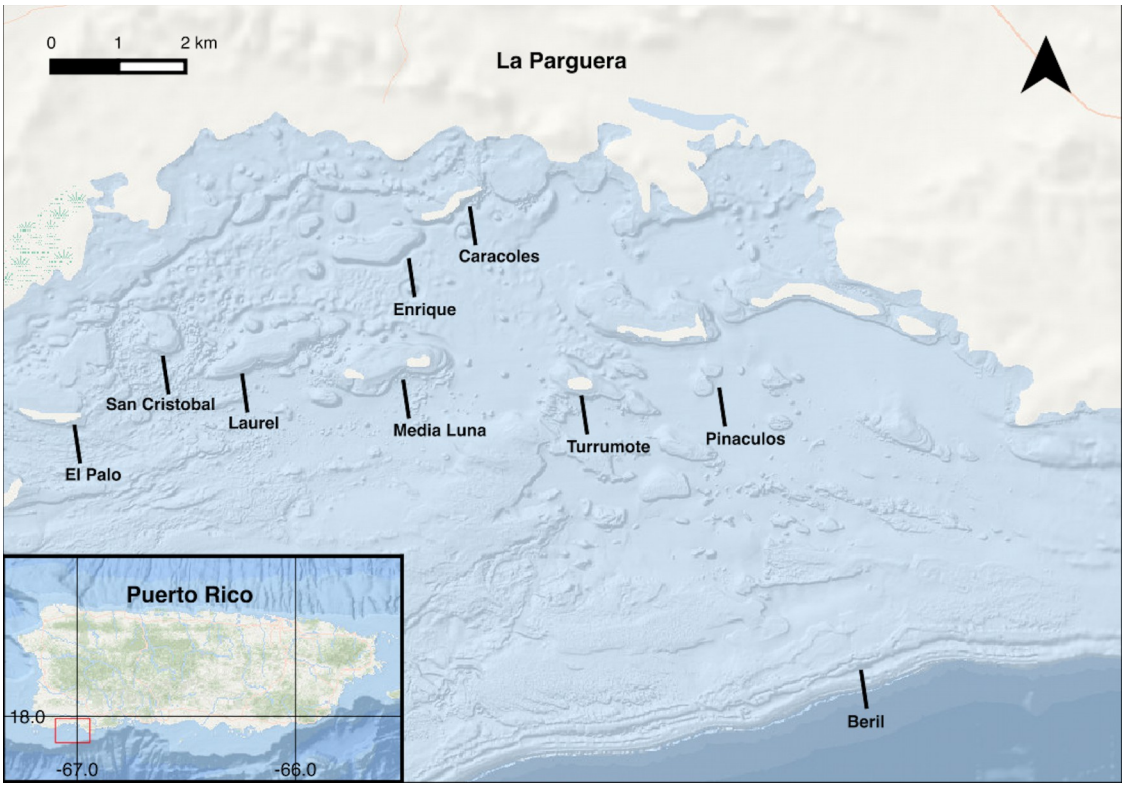

Fig. 2 Map of La Parguera, Puerto Rico with study sites. Image made with QGIS using NOAA's National Centers for Enviromental Information (NCEI) Multibeam Bathymetric Surveys Dataset.

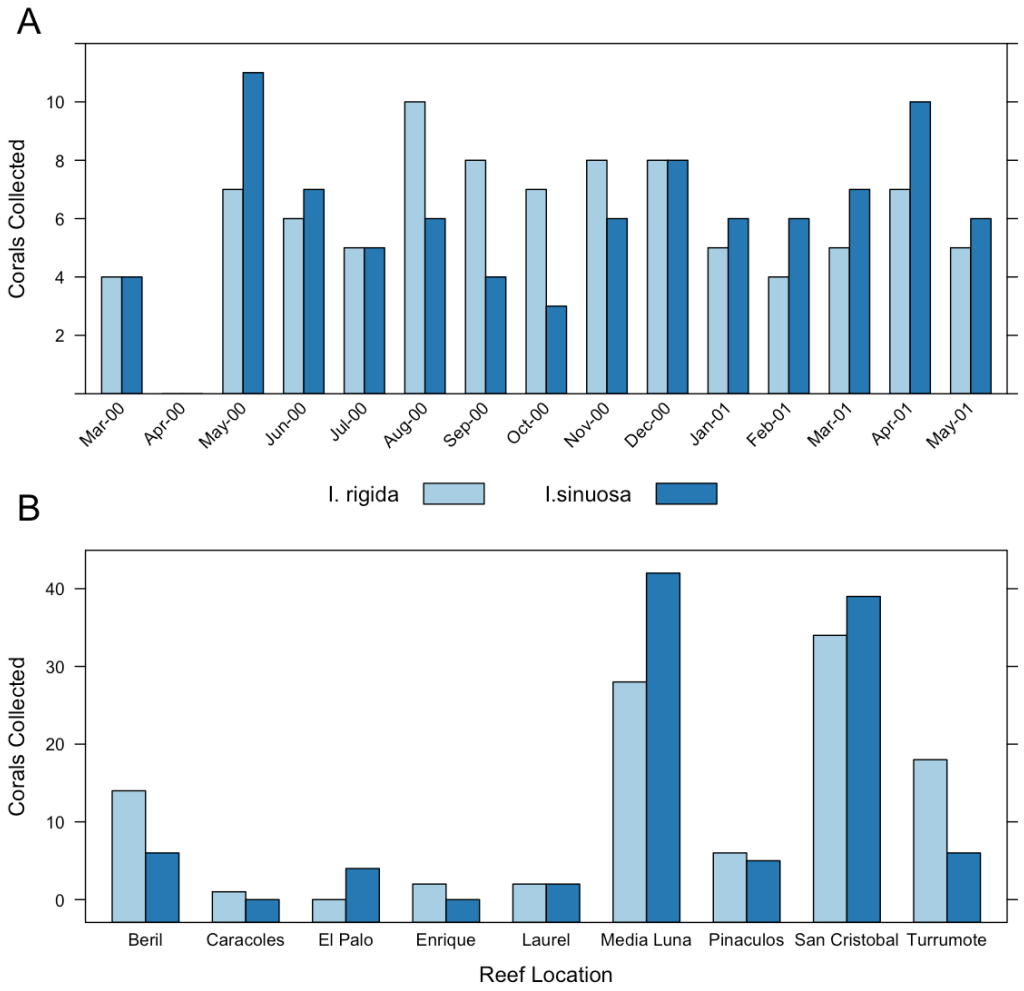

Fig. 3 (A) Number of samples collected per month **(B)** Number of samples collected per location.

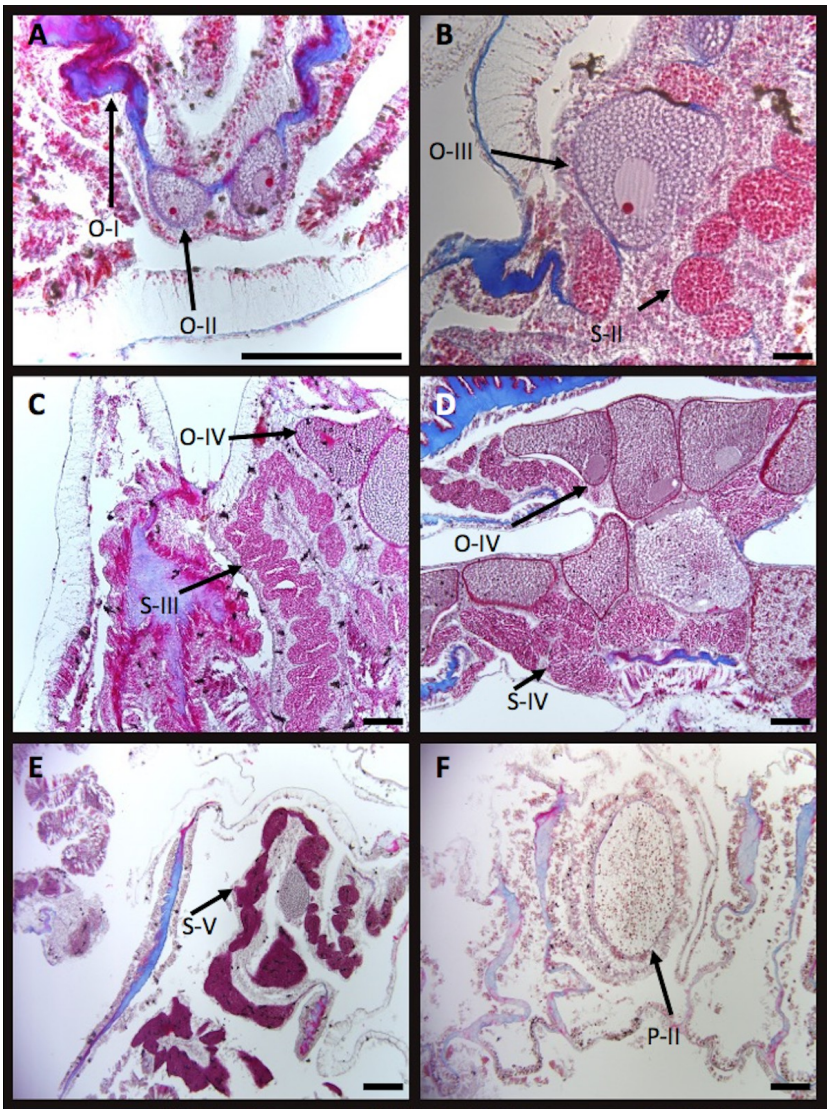

Fig. 4 Developmental stages of oocytes (O) and spemaries (S) in *I. sinuosa*. **(A)** stage I and II oocytes, **(B)** stage III oocytes, **(C)** stage II spermaries and stage IV oocytes, **(D)** stage IV oocytes and stage III spermaries, **(E)** stage IV oocytes and stage V spermaries, and **(F)** stage I planula. Reference bar measures $100\mu m^2$.

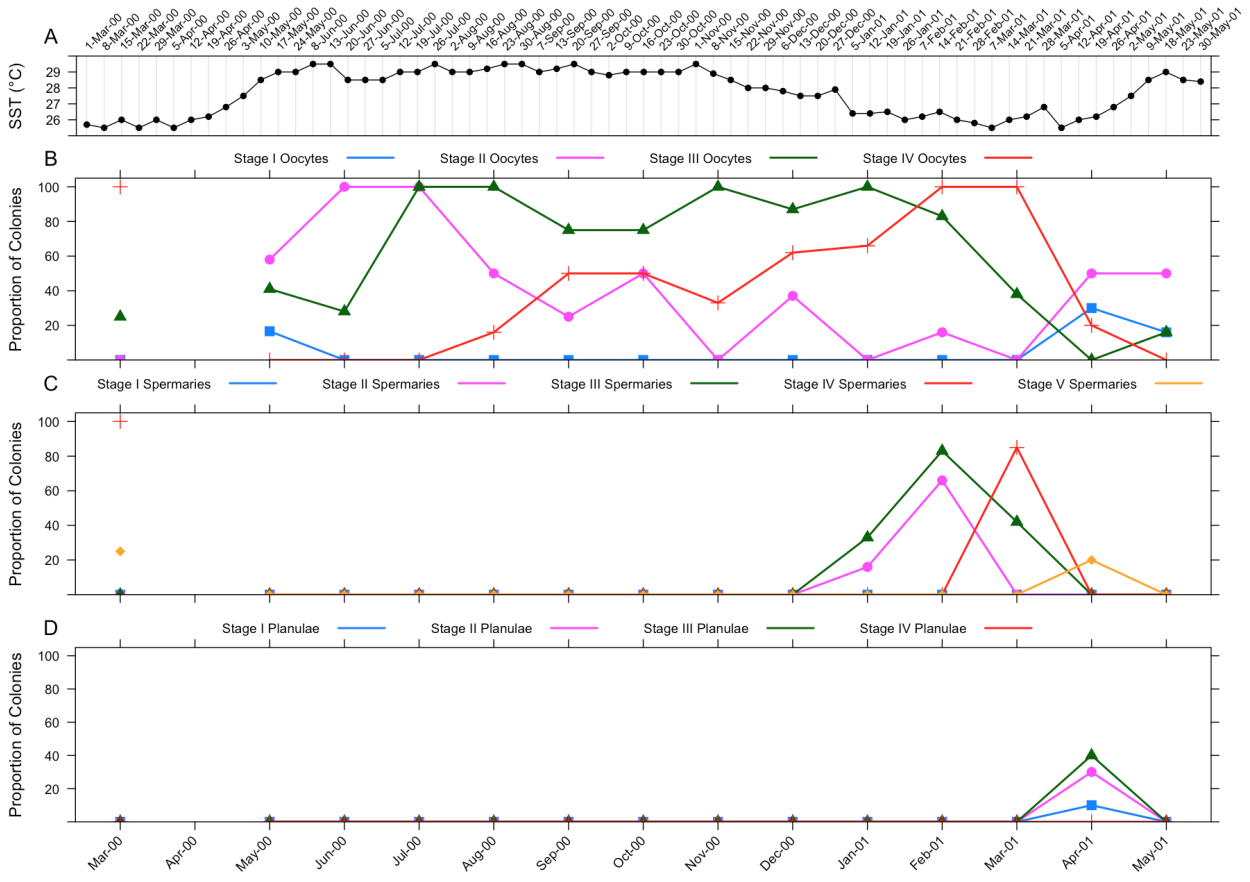

Fig. 5 (A) SST temperature ranges in La Parguera, Puerto Rico. Adjusted values of relative proportions of colonies of *I. sinuosa* in each gametogenetic stage of **(B)** oogenesis, **(C)** spermatogenesis, and **(D)** embryogenesis from March 2000 to May 2001.

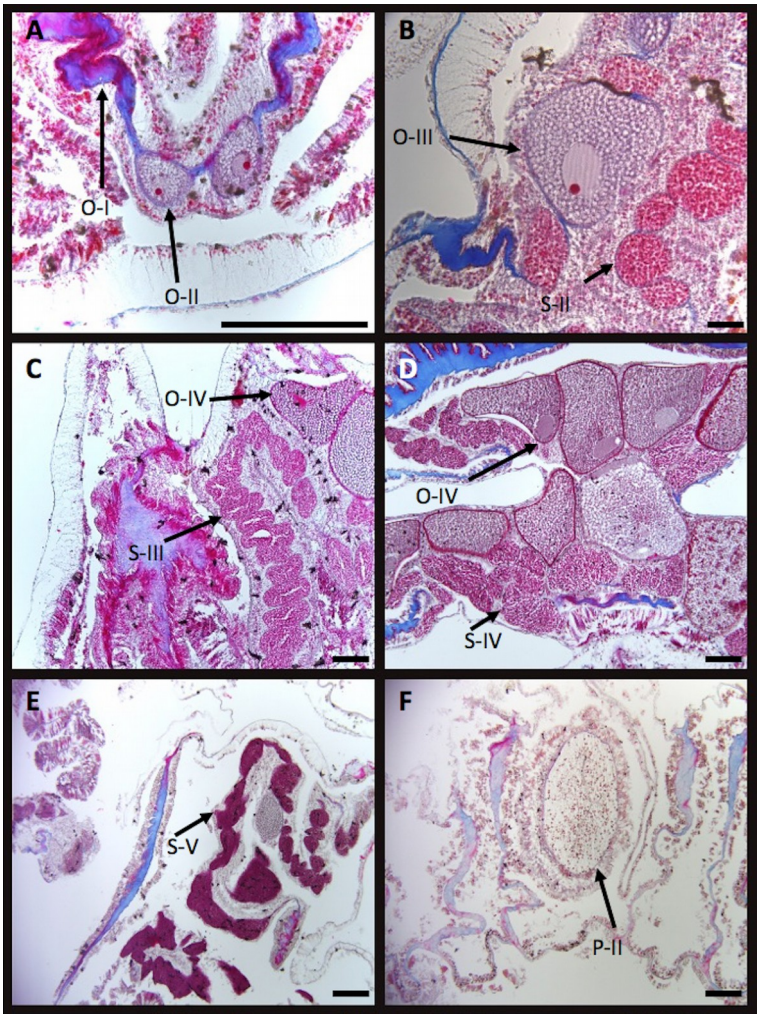

Fig. 6 Developmental stages of oocytes (O) and spemaries (S) in *I. rigida*. **(A)** stage I and stage II oocytes in the mesoglea, **(B)** Stage III oocytes and stage III spermaries, **(C)** stage III spermaries and stage IV oocytes, **(D)** stage IV oocytes and stage IV spermaries, **(E)** stage V spermaries, and (**F)** stage II planula. Reference bar measures 100µm^2.

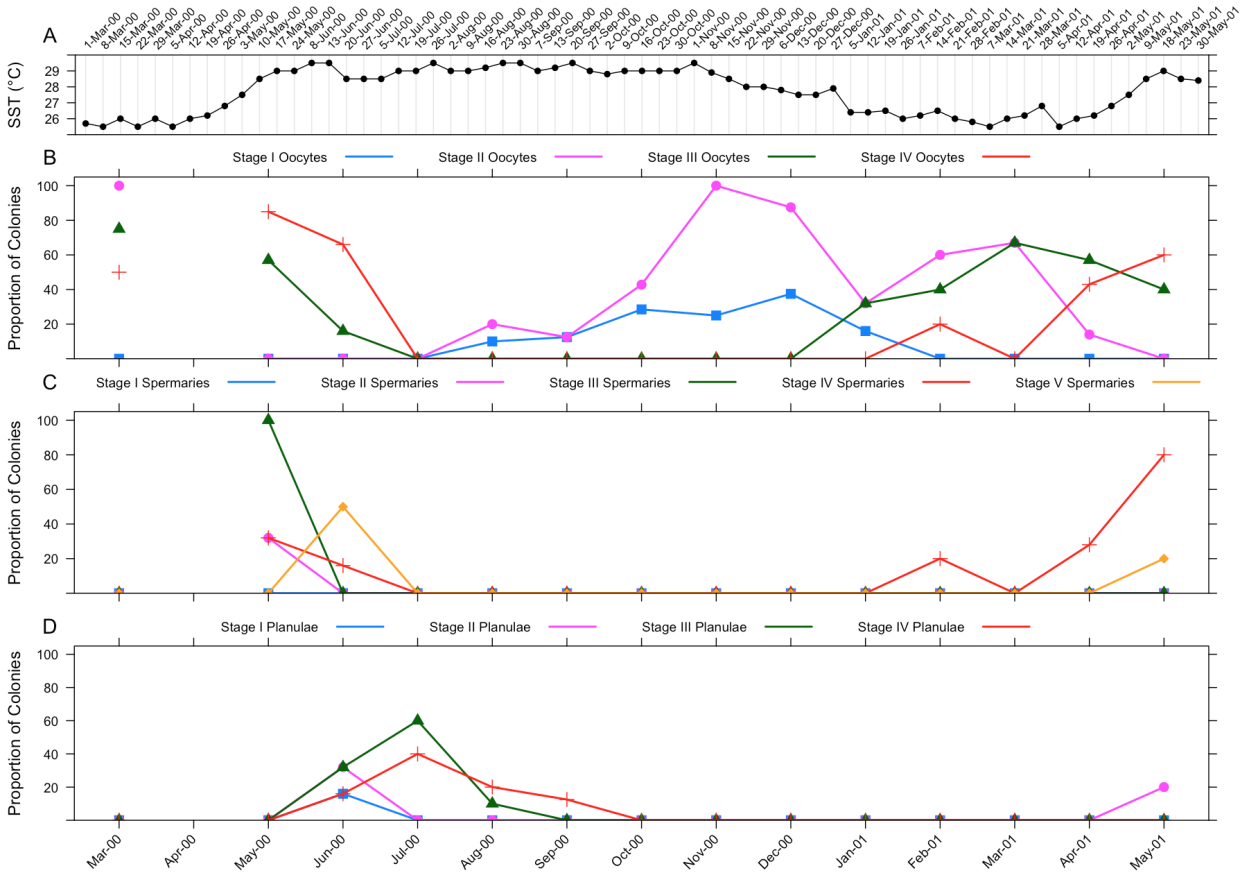

Fig. 7 (A) SST temperature ranges in La Parguera, Puerto Rico. Adjusted values of relative proportions of colonies of *I. rigida* in each gametogenetic stage of **(B)** oogenesis, **(C)** spermatogenesis, and **(D)** embryogenesis from March 2000 to May 2001.

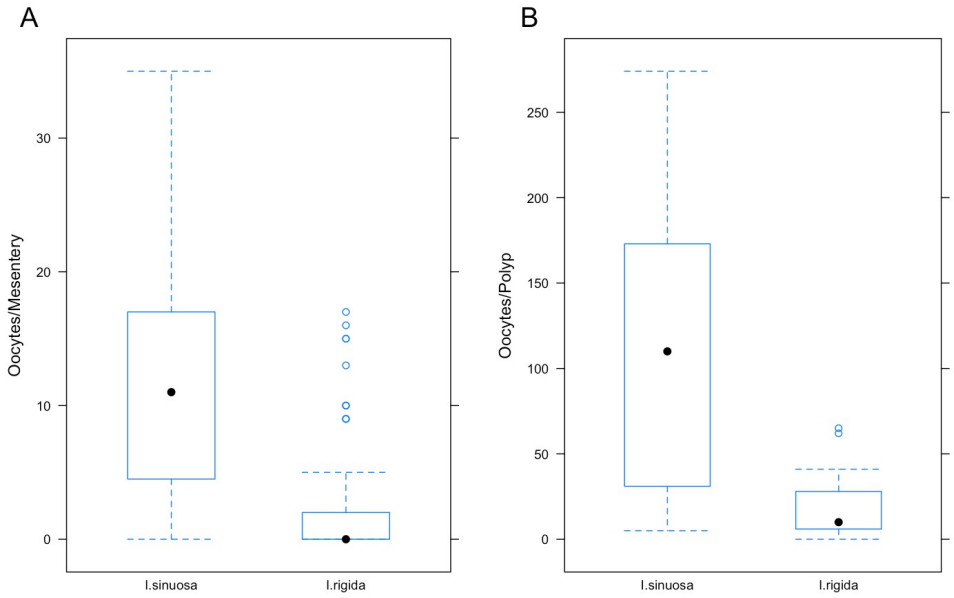

Fig. 8 (A) Average mesenterial (eggs/mesentery) fecundity and **(B)** polyp (eggs/polyp) fecundity in *I. sinuosa* and *I. rigida.* Error bars represent standard deviation.

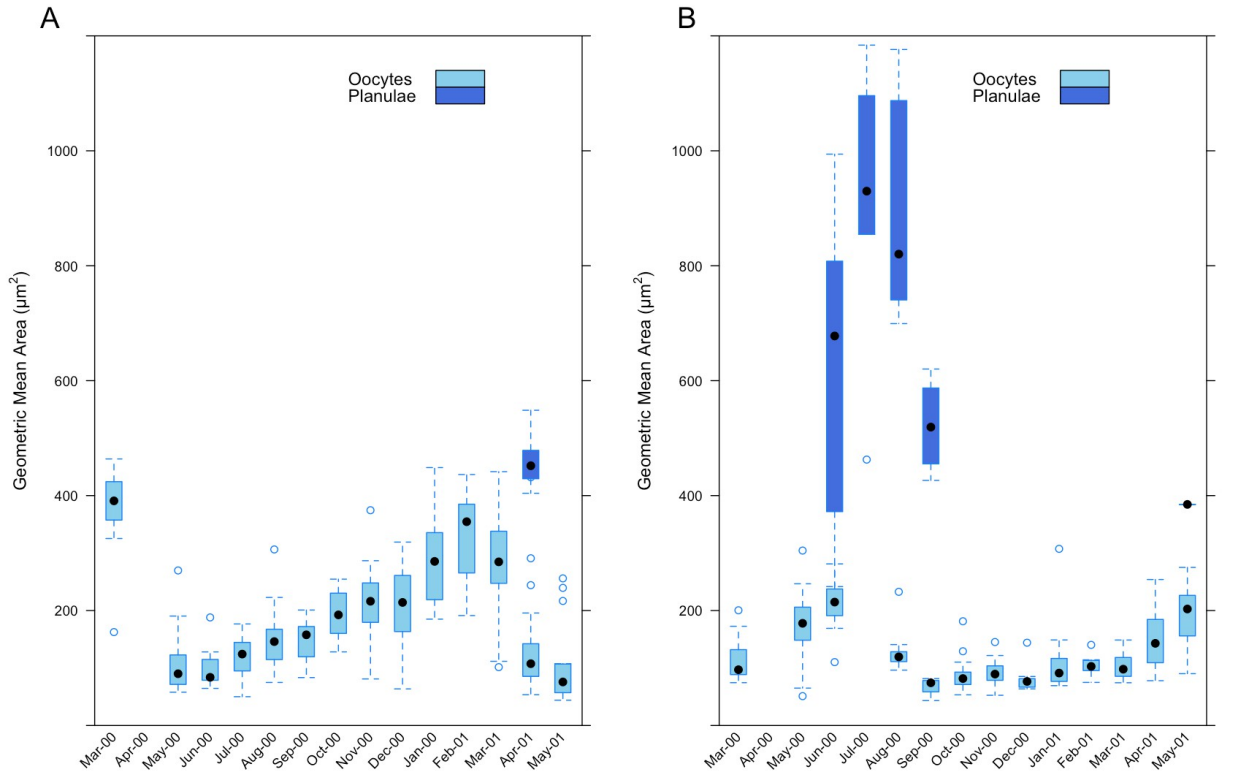

Fig. 9 Monthly geometric mean oocyte and planulae area in **(A)** *I. sinuosa* and **(B)** *I. rigida.*

Table 1 Comparison of reproductive characteristics of *Mussidae* (Clade XXI)

Subfamily	Genus	Species	Sexual Pattern	Mode of Development	Source
Mussinae	*Mussa*	*M. angulosa*	H		Steiner 1993
	Isophyllia	*I. rigida*	H	Brooding	**This study**
		I. sinuosa	H	Brooding	Duerden 1902; **This study**
	Mycetophyllia	*M. ferox*	G□H	Brooding	Szmant 1984; Szmant 1986; Morales 2006
		M. aliciae	H	Brooding	Morales 2006
		M. lamarckiana	H	Brooding	Morales 2006
		M. danaana	H	Brooding	Morales 2006
		M. reesi			
	Scolymia (Atlantic)	*S. cubensis*	H	Brooding	Weil unpublished data
		S. lacera	H	Brooding	Weil unpublished data
		S. wellsi	H	Brooding	Pires, Castro and Ratto 2000
Faviinae	*Favia (Atlantic)*	*F. fragrum*	H	Broadcast	Duerden 1902; Fadlallah 1983; Szmant 1986; Richmond and Hunter 1990; Soong 1991
	Colpophyllia	*C. amaranthus*	H	Broadcast	Weil unpublished data
		C. natans	H	Broadcast	Steiner 1995; Hagman et al. 1998; Boland 1998; Weil unpublished data
	Diploria	*D.labyrinthiformis*	H	Broadcast	Duerden 1902; Fadlallah 1983; Wyers et al. 1991; Weil and Vargas 2009
	Pseudodiploria	*D. clivosa*	H	Broadcast	Soong et al. 1991; van Veghel 1993; Weil and Vargas 2009
		D. strigosa	H	Broadcast	Szmant 1986; Richmond and Hunter 1990; Soong 1991; Steiner 1995; Weil and Vargas 2009
	Manicina	*M. areolata*	H	Brooding	Duerden 1902; Fadlallah 1983; Richmond and Hunter 1990; Johnson 1992
	Mussismilia	*M.hispida*	H	Broadcast	Neves and Pires 2002; Pires, Castro and Ratto 1999
		M. hartii	H	Broadcast	Pires, Castro and Ratto 1999
		M. brazilensis	H	Broadcast	Pires, Castro and Ratto 1999

468 *H* hermaphroditic, *G* gonochoric