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Research Article

Gametogenesis and Spawning of *Solenastrea bournoni* and *Stephanocoenia intersepta* in Southeast Florida, USA

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This study constitutes the first report of the gametogenic cycle of the scleractinian corals *Solenastrea bournoni* and *Stephanocoenia intersepta*. Tissue samples were collected near Ft. Lauderdale, Florida, USA between July 2008 and November 2009 and processed for histological examination in an effort to determine reproductive mode and potential spawning times. Both *S. bournoni* and *S. intersepta* are gonochoric, broadcast spawning species. Gametogenesis of *S. bournoni* began in April or May while *S. intersepta* had a much longer oogenic cycle that began in December with spermatogenesis beginning in July. Though spawning was not observed *in situ*, spawning was inferred from the decrease of late stage gametes in histological samples. In addition, histological observations of oocyte resorption and released spermatozoa were used to corroborate spawning times. Data indicate that *S. bournoni* spawns in September while *S. intersepta* spawns after the full moon in late August or early September.

1. Introduction

Reproduction of scleractinian corals is one of the most important processes influencing their continued existence [1]. Sexual reproduction is the first step in establishing new colonies necessary for repopulating degraded reefs. It creates genetic variability essential for adapting to changing environmental conditions. Information on the reproductive biology and ecology of corals is critical for understanding their distribution, evolutionary mechanisms [2, 3], and for management and restoration of damaged areas [4].

For broadcast spawning species, synchronous maturation and release of gametes is essential for successful fertilization [5]. Coral gametogenesis and spawning are thought to be driven by a number of environmental cues including sea temperature, day length, moonlight tidal cycles, and daylight cycles [6, 7], but the exact association remains unknown [1, 7, 8]. For a number of species, temperature defines the season and month of spawning and timing tends to correlate strongly with lunar phase [5]. Sunset time can define the hour of spawning [6, 9, 10] and tidal fluctuations may decrease water movement and lead to increased fertilization opportunities [11]. It is very likely that coral gametogenesis and spawning are not prompted by a single environmental cue, but rely on more than one environmental signal [12].

In the last few decades, there have been numerous studies on scleractinian coral reproduction [4, 7, 13–16]. About 60% of the approximate 60 known Caribbean species have been investigated [3]. Species of particular interest in southeast Florida include *Solenastrea bournoni* and *Stephanocoenia intersepta* because of their local abundance. *Stephanocoenia intersepta* and *Solenastrea bournoni* are common species found offshore in all three counties (Miami-Dade, Broward, and Palm Beach) and on all reef types (nearshore ridge complex, Inner reef, Middle reef, and Outer reef) within the southeast Florida region [17, 18]. These species are not as common in the rest of the Caribbean where *S. intersepta* generally comprises less than 5% of the coral population in areas such as the US Virgin Islands, Venezuela, Turks and Caicos, Mexico, Belize, and Bahamas and *S. bournoni* is even less abundant [19]. There is no information in the published literature on reproductive mode or spawning time of *S. bournoni*. *Stephanocoenia intersepta* has been reported to be a gonochoric species that has been observed broadcast

Table 1: Criteria for differentiating oocyte and spermary developmental stage based on Szmant-Froelich et al. [27].

Stage	Oocytes	Spermaries
I	Nucleus large, located in or adjacent to mesoglea	Small cluster of ≤10 cells surrounded by mesoglea
II	Accumulation of cytoplasm around nucleus, located in mesoglea	Larger cluster of ≥10 cells
III	Increased amount of cytoplasm, but no vitelline membrane	Cells closer together, central lumen developed
IV	Larger, elongated and presence of vitelline membrane	Cells very small, undergoing meiosis, tails in lumen

spawning in Bonaire 3–7 nights after the August full moon [16] and in the Gulf of Mexico from 6 to 11 nights after the August or September full moon between the hours of 19:30 and 20:00 [9, 20]. Females of this species have been observed releasing a high percentage of fertilized eggs [20, 21] and it has been proposed that eggs are fertilized in the tentacles just prior to release [21]. Cycles of oogenesis and spermatogenesis have not been previously described for these two species. The purpose of this study was to document the gametogenic cycle of *S. bournoni* and *S. intersepta* and to determine their spawning times in southeast Florida.

2. Materials and Methods

2.1. Collection and Histological Processing. *Solenastrea bournoni* and *S. intersepta* were sampled twice per month from July 2008 through July 2009 and weekly in August and September 2009. During each collection date, five colonies of *S. bournoni* and *S. intersepta* were sampled. Additionally, five *S. bournoni* samples were collected twice per week in October and November 2009. Colonies were sampled in southeast Florida near Ft. Lauderdale (26°03′N to 26°19′N) in 4 to 18 m depth. Tissue samples measuring approximately 6–12 cm^2 in size were removed using a hammer and chisel or a 2.5 cm steel core. All samples were taken from the middle of the colony to avoid less fecund edges [22–24]. Only colonies that had a diameter of over 10 cm were sampled to ensure sexual maturity [14, 24–26] and each colony was sampled only once. Epoxy was placed on the exposed skeleton where tissue was removed to minimize potential settlement of algae and boring organisms. Collection methods caused minimal damage to *S. bournoni* colonies since tissue completely regrew over the sample area within 6 months. However, *S. intersepta* had much slower regeneration rates and showed minimal regrowth over the sampled areas during the study period. Collected samples were fixed immediately in 10% aqueous zinc-buffered formalin (Z-fix) for 24 hours. Tissue samples were decalcified using a buffered 10% hydrochloric acid solution, dehydrated using a series of ethanol and xylene, infiltrated with paraffin, and embedded in paraffin blocks.

Serial sections of 5 μm were cut with a microtome at three depths of the tissue and placed on slides. The first serial sections were taken at a location just below the actinopharynx, and subsequent sections were taken approximately 0.15–0.3 mm and 0.45–0.6 mm below the actinopharynx. Two replicate slides were made at each of the three depths, yielding 6 slides for each sample. Three of these slides, one at each depth, were stained with Heidenhain's azocarmine aniline blue to highlight reproductive structures. The rest were stained with Harris' hematoxylin and eosine (H&E) in order to confirm the presence of released spermatozoa in which the nuclei stain dark purple. All staining characteristics described are with Heidenhain's azocarmine aniline blue unless otherwise noted.

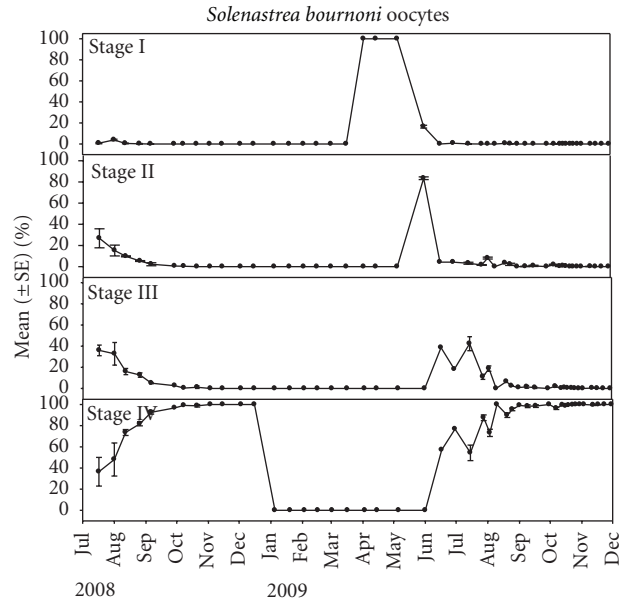

Figure 1: *Solenastrea bournoni* mean (±SE) percentage of oocytes per colony quantified through histological examination.

2.2. Quantification of Gametes and Fecundity. Gamete developmental stages were determined from the slide that contained the most gametes which was usually the deepest slide. Criteria determining gamete developmental stage (Table 1) were modified from Szmant-Froelich et al. [27]. Using the slide with the most gametes, the percentage per colony of each gamete stage was calculated per sampling date using the total number of gametes in 7–10 polyps per colony. Resorption and released spermatozoa percentages per sampling date were calculated based on the number of mesenteries of 7–10 polyps per colony that contained resorbed oocytes or spermatozoa released from spermaries. Polyp fecundity was calculated for every female colony sampled during the month prior to spawning. Fecundity was calculated by multiplying the mean number of mature (stage IV) oocytes in cross sections of ten polyps per colony and the mean number of mature oocytes in longitudinal sections of 10 mesenteries, yielding mean stage IV oocytes

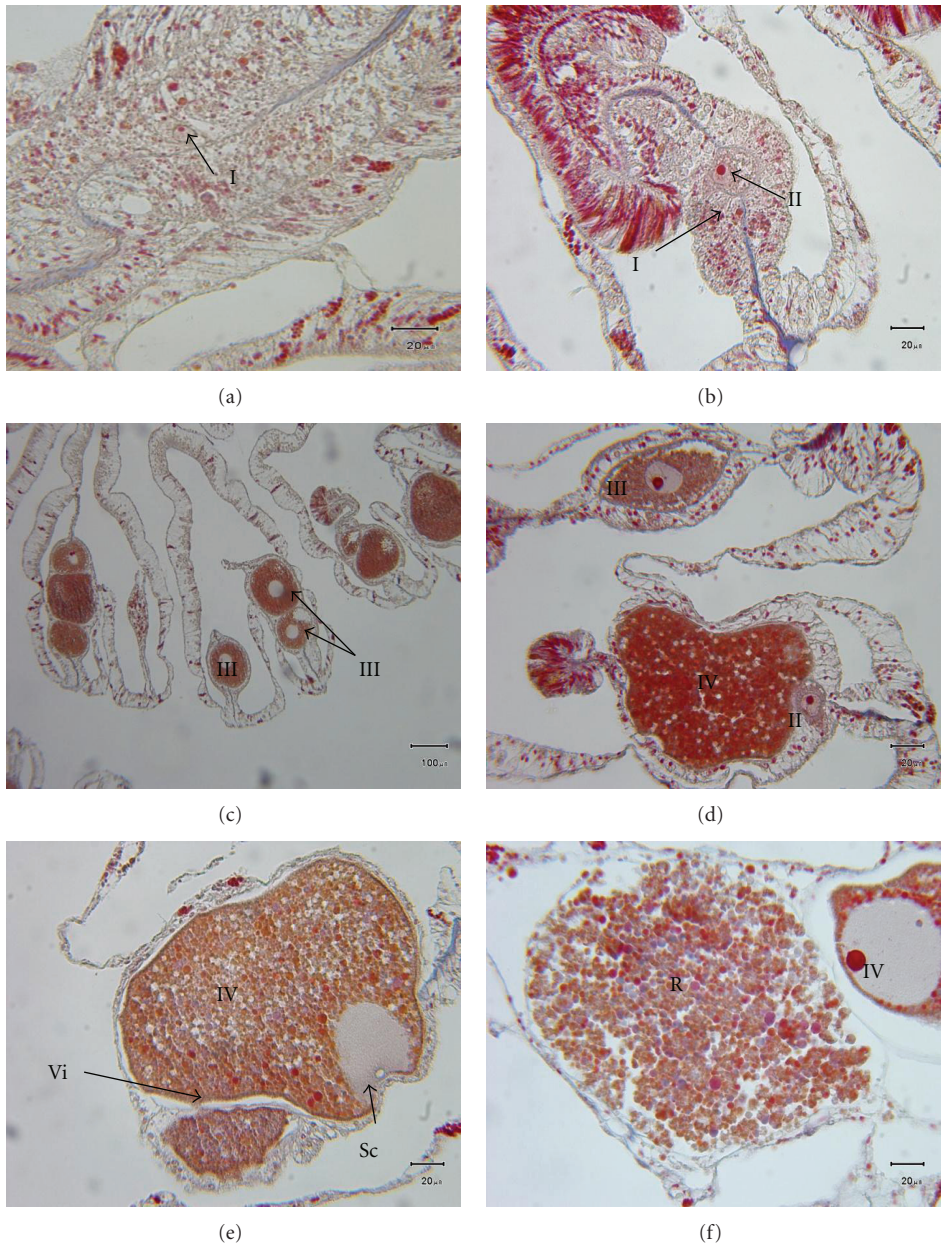

Figure 2: Photomicrographs of *Solenastrea bournoni* oocyte stages I–IV (defined by Table 1). (a) Stage I oocyte; (b) stages I and II oocytes; (c) stage III oocytes; (d) stages II, III and IV; (e) stage IV oocytes, Vi = vitelline membrane, Sc = scalloping of nucleus; (f) stage IV oocyte adjacent to oocyte resorption (R). All scale bars 20 μm except for (c) which is 100 μm.

per polyp. For description of developmental stages, mean oocyte diameters were measured from multiple colonies over several sampling dates when abundance was highest. Mean abundance of stage IV oocytes per colony was calculated and compared to environmental factors including moon phase and temperature. Chi-squared tests were used to determine sex ratio significance.

2.3. Environmental Data. Temperature loggers were deployed at 10 meters depth where most coral samples were collected. Regression analyses were preformed to examine the relationship between oocyte abundance and temperature. Stage IV oocyte abundance was plotted with moon phase to determine if spawning times could be linked to lunar cycles.

3. Results

3.1. Solenastrea bournoni. *Solenastrea bournoni* is a gonochoric species with a 1 : 1 sex ratio ($\chi^2 = 0.157$, df = 1, $P = 0.692$). A total of 112 females and 118 males were sampled, and seven colonies did not have gametes and

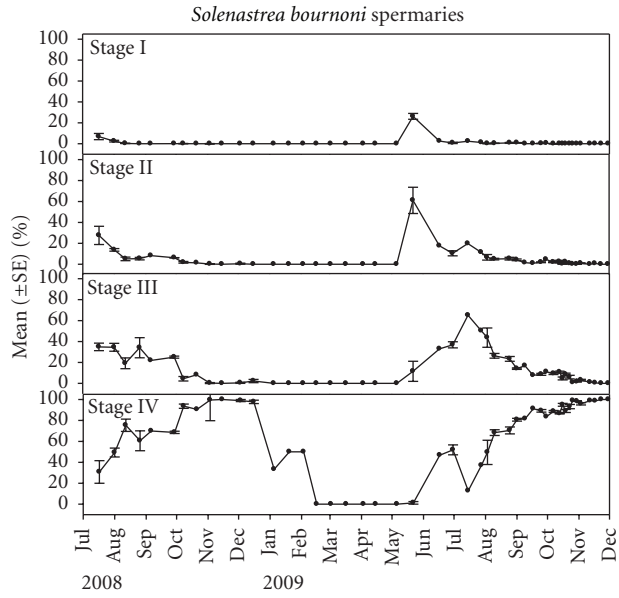

Figure 3: *Solenastrea bournoni* mean (±SE) percentage spermary stages per colony quantified through histological examination.

could not be determined to be male or female. Out of 237 colonies sampled, there were two male colonies that contained a single mature oocyte in the same mesentery that contained spermatocytes and one male colony that contained two oocytes in separate polyps surrounded by mature spermatocytes. No larvae were seen in any colony, and late stage oocytes did not contain zooxanthellae. Fecundity was calculated as 166.34 ± 42.00 (SE) oocytes per polyp (n = 8 colonies) in 2008 and 305.33 ± 78.48 oocytes per polyp (n = 10) in 2009.

3.1.1. Gametogenesis. Oogenesis in *Solenastrea bournoni* began in April, and stage I oocyte abundance peaked from April to early May (Figure 1). Mean diameter of stage I oocytes observed during their peak abundance was 25.24 ± 0.98 μm and ranged in size from 16 to 30 μm (n = 26 oocytes). Early stage I oocytes were located in the gastrodermal layer and migrated into the mesoglea (Figures 2(a) and 2(b)). They were characterized by large nuclei in relation to the amount of cytoplasm. Nuclei stained light pink to grey with a magenta nucleolus, and the cytoplasm stained light grey.

Stage II oocytes were first observed in early June (Figure 1) but may have developed earlier (no females were collected in late May). Highest percentages per colony were seen in early June. Mean diameter of randomly chosen stage II oocytes from June samples was 57.32 ± 2.83 μm and ranged from 35 to 98 μm (n = 30). These larger oocytes were located in the mesoglea and stained light grey with a light pink nucleus and magenta nucleolus (Figure 2(b)). The size of cytoplasm was greater than the nucleus.

Stage III oocytes began to appear in mid-June, and abundance peaked in mid-July 2008 and in early August 2009 (Figure 1). Stage III oocytes measured during this period were larger than stage II with a mean diameter of 102.12 ± 3.65 μm and a range of 69–149 μm (n = 30). Stage III oocytes stained a darker pink in color and had a centrally located nucleus (Figures 2(c) and 2(d)).

Stage IV oocytes developed by mid-June, peaked in August, and continued to comprise a high percentage of total oocytes through the end of sampling in late November (Figure 1). Mean diameter of stage IV oocytes was 225.59 ± 10.18 μm and ranged from 153 to 341 μm (n = 30). Stage IV oocytes were characterized by their large size and dark vitelline membrane (Figure 2(d)). Depending on the section, the nucleus had usually migrated to one side, and the vitelline membrane sometimes had a scalloped appearance (Figure 2(e)). Stage IV oocytes also had an increased amount of lipid vacuoles that stained orange, pink, magenta, and light purple.

Resorption of oocytes was observed throughout most of the year in 2009 although in small percentages. In early resorption (Figure 2(f)), the vitelline membrane broke apart, and lipid vacuoles were clumped together. Lipid vacuoles slowly dispersed into the gastrodermis and degraded. Resorption after February was seen in less than 1% of all mesenteries examined. In July, there was a low occurrence of resorption (4.46% of mesenteries in all colonies sampled that month), and in early October one colony showed resorption of oocytes in 20% of its mesenteries. Higher levels of resorption were observed through the end of sampling in late November.

Spermatogenesis began rapidly in May with all stages appearing in the same two-week period (Figure 3). Stage I and stage II spermaries stained light pink and were differentiated by the number of cells (Figures 4(a) and 4(d)). Stage I spermaries were characterized by fewer than 10 small, loosely clustered cells located in the mesoglea, and stage II spermaries contained more than 10 cells. Both stage I and stage II spermaries had the highest percentages in May (Figure 3).

Stage III spermaries peaked in mid-July (Figure 3) and were comprised of larger and darker cells that were located closer together around a centrally located lumen (Figures 4(b) and 4(d)). The highest percentages of stage IV spermaries were in October, and they were observed through February (Figure 3). The magenta cells were very small, and spermatozoa tails were lined up in the lumen (Figures 4(c) and 4(d)).

Low percentages of released spermatozoa were observed in the gastrodermis during many of the sampling dates (Figures 4(d) and 4(e)). Small spikes in percentage of released spermatozoa occurred in mid-August and October 2008 followed by the highest percentage observed in November. In 2009, released spermatozoa were seen in variable percentages between colonies starting in January. Percentage of released spermatozoa decreased greatly in May, but they were still observed in small percentages until July. Then percentages increased at the end of September, continuing through November to near 100%. In stains of hematoxylin and eosine (H&E), the heads of the spermatozoa stained dark purple shortly after being released (Figure 4). Months later,

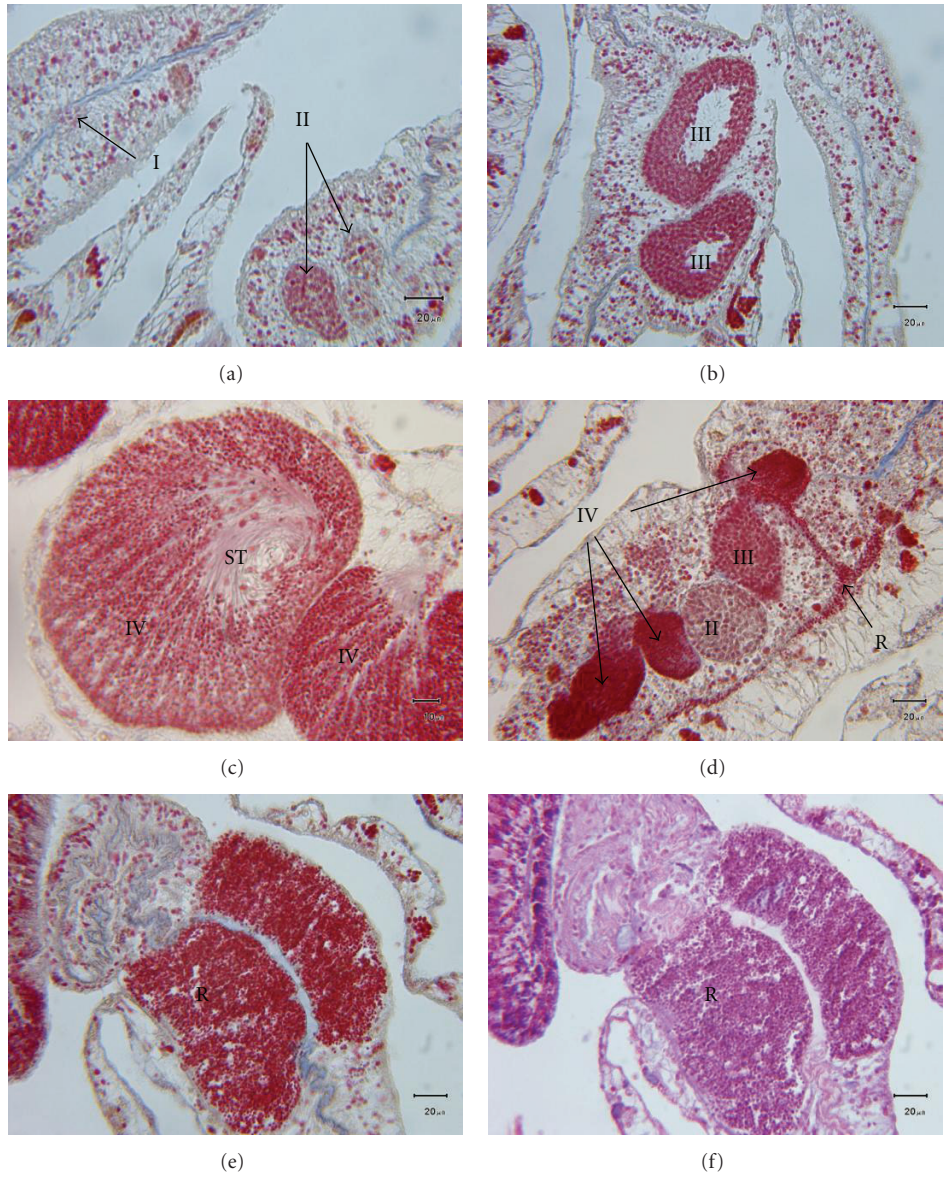

Figure 4: Photomicrographs of *Solenastrea bournoni* spermary stages I–IV (defined by Table 1). (a) Stages I and II spermaries; (b) stage III spermaries; (c) stage IV spermaries, ST = spermatozoa tails lined up; (d) stages II, III, and IV, R = spermatozoa being released into gastrovascular cavity; (e) released spermatozoa (R) stained with Heidenhain's azocarmine aniline blue; (f) released spermatozoa (R) stained with H&E. All scale bars 20 μm except for (c) which is 10 μm.

residual spermatozoa did not stain purple, indicating they had degraded and were no longer viable.

3.1.2. Lunar Periodicity. Mean abundance of stage IV oocytes per colony was plotted with lunar phase (Figure 5). Peak abundances of stage IV oocytes occurred in late August and early September and experienced the largest decrease between the full and new moon of September. Smaller decreases in abundance were also observed between the full and new moons of October though few female colonies were sampled before the October 2008 full moon and after the October 2009 full moon.

3.1.3. Temperature. Mean stage IV oocyte abundance tracked mean daily water temperatures (Figure 6) with oocyte abundance decreasing and increasing in unison with temperature. In 2009, peaks in oocyte abundance in July, September, and August occurred after peaks in temperature. To test this correlation, a simple linear regression was performed plotting all stages of oocyte abundance against temperature (Figure 7). A significant relationship was found between oocyte abundance and temperature ($R^2 = 0.67$, $P < 0.001$), so the same analysis was performed for all stages of spermary abundance (Figure 8). A significant relationship was also found between spermary abundance and temperature ($R^2 = 0.65$, $P < 0.001$).

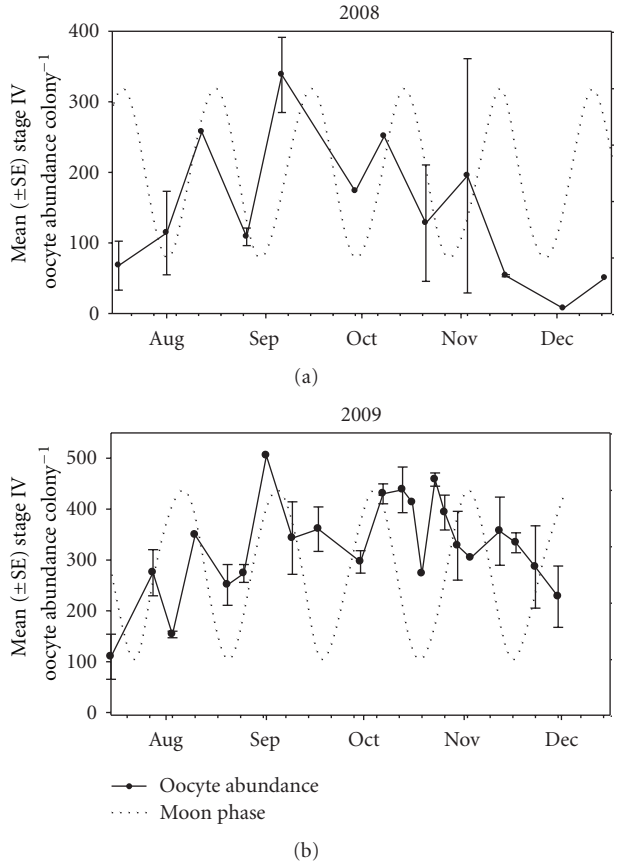

Figure 5: *Solenastrea bournoni* mean (±SE) abundance of stage IV oocytes per colony at each sampling date with moon phases for years 2008 and 2009; full moons represented by peaks in moon phase line.

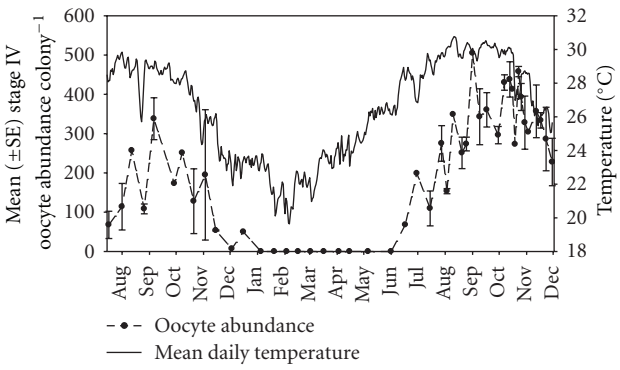

Figure 6: *Solenastrea bournoni* mean (±SE) abundance of stage IV oocytes per colony at each sampling date with temperature for sampling dates 2008 through 2009.

3.1.4. Predicted Spawning Times. Results indicate that *Solenastrea bournoni* spawned after the full moon of September. In 2008, the greatest decrease in mean oocyte abundance was observed between 6 and 29 September, from 338 to 173 oocytes per colony. The full moon occurred on September 15. Oocyte resorption was first observed in October in

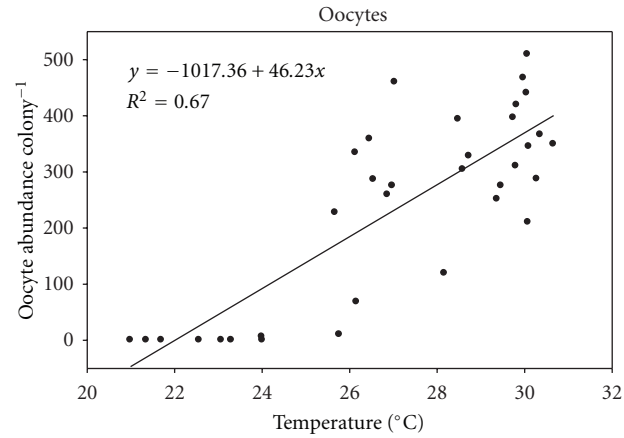

Figure 7: Simple regression of temperature and *Solenastrea bournoni* oocyte abundance, including all stages per colony at each sample date. $R^2 = 0.67$, $P < 0.001$.

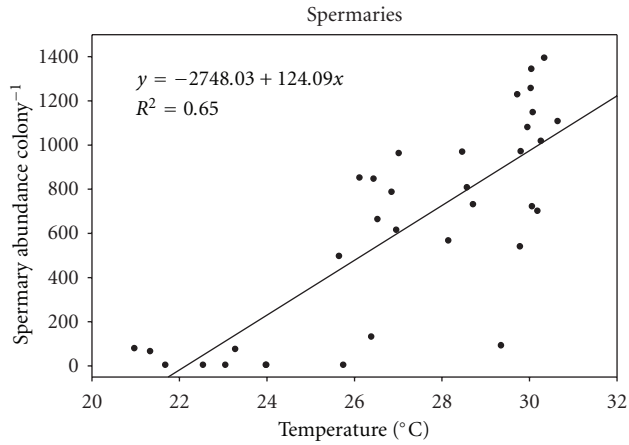

Figure 8: Simple regression of temperature and *Solenastrea bournoni* spermary abundance, including all stages per colony at each sample date. $R^2 = 0.65$, $P < 0.001$.

low percentages, and percentages increased in November. Released spermatozoa were observed in August but were not present again until October. A greater amount of oocyte resorption and released spermatozoa in October indicates that the main spawning event took place before October. In 2009, the highest peak in mean abundance of stage IV oocytes was on 9 September with 505 oocytes per colony. A full moon occurred on September 4, and mean abundance of stage IV oocytes decreased after 9 September. Oocyte resorption also started in October 2009. The amount of released spermatozoa was moderate on 17 September, but increased through December of 2009. Unfortunately, the fact that only one of the five colonies sampled after the potential spawning time in 2008 was female and only one of the five colonies sampled before the potential spawning in 2009 was female precludes statistical analysis of significant decreases in oocyte abundance to support the proposed spawning period. However a similar decrease in mature oocyte abundance after the full moon of September in both years, coupled

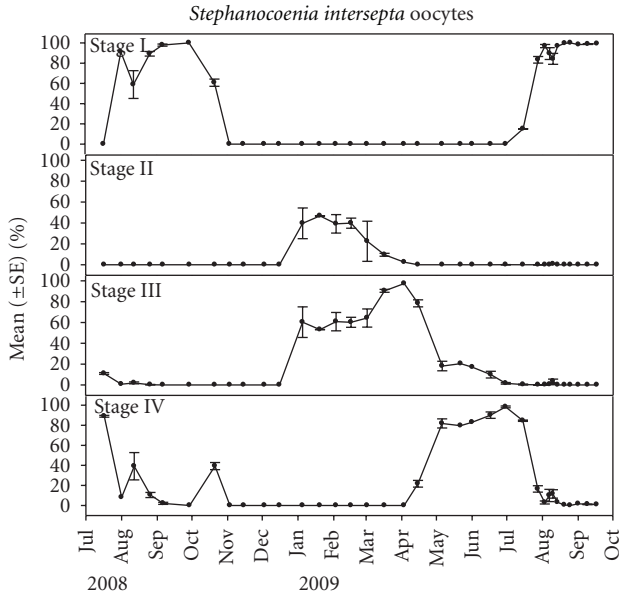

Figure 9: *Stephanocoenia intersepta* mean (±SE) percentages of oocyte per colony quantified through histological examination.

with observed oocyte resorption and increases of released spermatozoa in October, indicates the main spawning event likely occurred after the full moon of September.

3.2. Stephanocoenia intersepta. No cases of hermaphroditism were observed in 181 colonies sampled over 15 months. Sixty-five colonies were found to contain no gametes. No sex could be assigned to 31 of these 65 colonies because 11 had no gametes during the reproduction period and 20 were sampled during the winter nonreproduction period. The remaining 34, sampled from January to June, were assumed to be males that had not yet developed gametes since spermatogenesis was not observed until July. Sex ratio was 1 : 1 (78 females, 72 males, $\chi^2 = 0.24$, df = 1, $P > 0.01$). Female fecundity of *S. intersepta* was 64.51 ± 12.04 oocytes per polyp in 2008 ($n = 8$) and 92.67 ± 9.27 oocytes per polyp in 2009 ($n = 19$).

3.2.1. Gametogenesis. Oocytes were observed in samples of *S. intersepta* from December through October while spermatocytes were present from late July through October. Resorption of oocytes was present for 2 to 3 months in September to early November. Released spermatozoa were present in September and October, but by mid-November, no gametes or remnant gametes were seen.

Stage I oocytes were first observed in mid-December in a longitudinal section of one colony but were not seen in any cross sections which were used to quantify gamete stages. Stage I oocytes peaked in percentage in January and were observed through August 2009 in low percentages (Figure 9). Stage I oocytes randomly chosen during January through August had a mean diameter of 15.81 ± 0.84 μm and ranged from 9.9 to 28.86 μm ($n = 30$ oocytes). These oocytes were found in the gastrodermis adjacent to mesoglea or in the mesoglea (Figure 10(a)). They had large nuclei in relation to the amount of cytoplasm, and the nuclei stained pink with a magenta nucleolus. If cytoplasm was seen, it was light pink or grey in color.

Stage I oocytes developed into stage II oocytes as early as December but were seen only in a longitudinal section of one colony. The highest percentage of Stage II oocytes was observed in April (Figure 9). Stage II oocytes randomly chosen from all dates observed had a mean diameter of 47.81 ± 2.09 μm, and they ranged in size from 31.33 to 63.71 μm ($n = 30$). Nuclei stained grey or light pink, and cytoplasm stained grey. Stage II oocytes were larger with the amount of cytoplasm greater than the nucleus, and they resided in the mesoglea (Figure 10(b)).

Stage III oocytes developed in April, and highest percentages were present in late June (Figure 9). The mean diameter of Stage III oocytes was 140.62 ± 5.69 μm and ranged from 79.86 to 206.3 μm ($n = 32$). Cytoplasm stained light pink, and oocytes usually had a centrally located nucleus (Figure 10(c)).

Stage IV oocytes first developed in mid-July, peaked in percentage in August, and declined after September (Figure 9). Stage IV oocytes randomly chosen from July through September had a mean diameter of 318.84 ± 13.78 μm and ranged from 184 to 435.2 μm ($n = 30$). The lipid vacuoles in the cytoplasm were more defined than they were in stage III and stained pink (Figure 10(d)). The vitelline membrane stained blue to purple and appeared scalloped in late stage IV oocytes (Figure 10(d)).

In 2008, resorption of *S. intersepta* oocytes occurred from early September through late October with no signs of resorption in November. In 2009, oocyte resorption was seen in low percentages in late July through early September, but in mid-September, oocyte resorption was at 100% in all mesenteries.

Stage I spermaries formed in late July, and the highest percentage was seen in August (Figure 11). Stage I spermaries were characterized by a small cluster of less than 10 loosely packed cells that stained light pink and were found in the mesoglea (Figure 12(a)). Stage II spermaries were observed in late July to early August with the highest percentages occurring in September 2008 and late August 2009 (Figure 11). Stage II spermaries stained light pink and consisted of more than 10 loosely packed cells (Figure 12(b)). Stage III spermaries were first observed in late July to early August, and peak percentages occurred in August (Figure 11). Stage III spermaries were larger with more compact, darker staining cells and had a centrally located lumen (Figure 12(c)). Stage IV spermaries were first observed in August, and the highest percentages occurred in early August 2008 and early September 2009 (Figure 11). Spermatocytes of stage IV spermaries stained dark magenta with pink staining tails (Figure 12(d)).

Released spermatozoa appeared in late September 2008 and were at their highest percentage in late October (Figure 11). In 2009, spermatozoa were observed outside of the mesoglea in early September, and in mid-September (Figures 12(e) and 12(f)) they were observed in almost at 60% of all mesenteries counted.

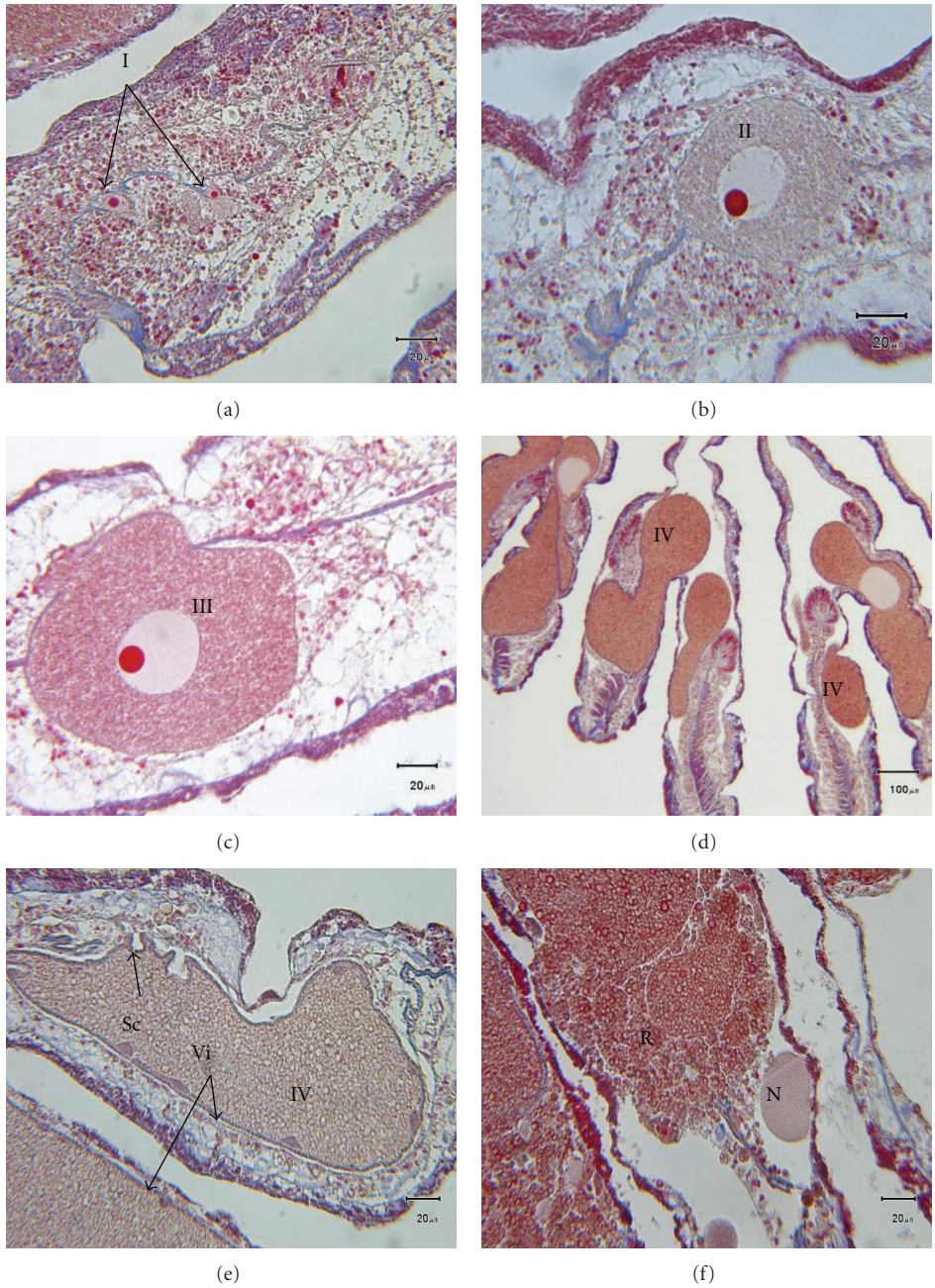

Figure 10: Photomicrographs of *Stephanocoenia intersepta* oocyte stages I–IV (defined by Table 1). (a) Stage I oocytes; (b) stage II oocyte; (c) stage III oocyte; (d) stage IV oocytes; (e) stage IV oocyte, Vi = vitelline membrane, Sc = scalloping of vitilline membrane; (f) oocyte resorption (R), N = Nucleus. All scale bars 20 μm except (d) which is 100 μm.

3.2.2. Lunar Periodicity. *Stephanocoenia intersepta* mean abundance of stage IV oocytes per colony on each sampling date was plotted with lunar phase (Figure 13). In 2008, there was a decrease in abundance between 26 August and 6 September which occurred after the full moon of 16 August. A decrease in stage IV oocytes occurred after 9 September 2009 following the full moon on 4 September.

3.2.3. Temperature. Oogenesis began during a time of cooler water temperatures in December. Abundance of all spermary stages was plotted against temperature, and a linear regression analysis indicated a very low correlation that was not significant ($R^2 = 0.09$, $P = 0.17$). However maximum abundance of late stage gametes occurred during the warmest time of the year.

3.2.4. Predicted Spawning Times. In 2008, highest abundance of stage IV oocytes was 10 days after the full moon of 16 August. Results of a Mann-Whitney rank-sum test indicated a significant difference ($P < 0.013$) in abundance of stage

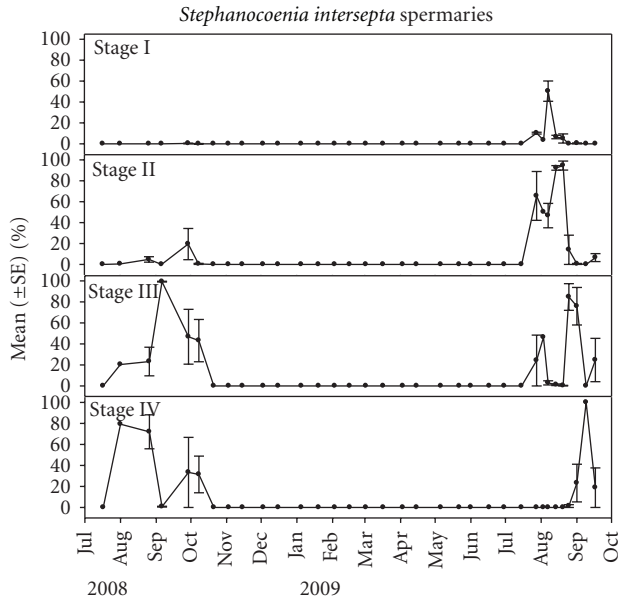

Figure 11: *Stephanocoenia intersepta* mean (±SE) percentages of spermaries per colony quantified through histological examination.

IV oocytes between colonies collected on 26 August and 6 September 2008, supporting the idea that *S. intersepta* spawned at least 10 days after the August 2008 full moon. In 2009, the greatest decrease in abundance of stage IV oocytes occurred between 9 September and 17 September. Unfortunately, only one female colony was sampled on 17 September which was the last sampling date. This colony showed oocyte resorption in 100% of all mesenteries examined. The four male colonies sampled on this date released spermatozoa in a mean of 60% of examined mesenteries, indicating a spawning event likely occurred before this date. These data indicate that *S. intersepta* spawned between 9 and 17 September 2009, following the full moon of 4 September.

4. Discussion

Gametogenic cycles of *S. bournoni* and *S. intersepta* differed greatly even though they likely spawned during similar times of the year. Most Caribbean broadcast spawning coral species studied to date begin gametogenesis a few months after spawning [24]. *Stephanocoenia intersepta* followed this pattern while *S. bournoni* did not. Gametogenesis of *S. bournoni* is similar to the Caribbean gonochoric broadcast spawning species *Oculina varicosa* which starts gametogenesis in early summer (*S. bournoni* starts in spring) and spawns in late summer and fall [8] as *S. bournoni* does. No reports of other Caribbean broadcast spawning species that begin gametogenesis in spring were found, indicating that this is an unusual occurrence.

Gametogenesis was more rapid in *S. bournoni* than in *S. intersepta*. Oogenesis in *S. bournoni* took less than 3 months (from early April to late June) to produce fully developed oocytes, but oocytes continued to develop for three more months through mid-October. The oogenic cycle of *S. intersepta* began in December, and oocytes slowly matured over 9 months. Spermaries developed rapidly for both species. No spermaries were seen in *S. bournoni* in early May. However, all stages were observed only 12 days later. *Stephanocoenia intersepta* spermaries took only one month to mature after spermatogenesis began in mid-to late July.

Temperature has been thought to play a potential role in inducing gametogenesis [28], and many Caribbean broadcast spawning species release their gametes during the highest annual water temperatures [20, 24]. Gametogenesis in *S. bournoni* was significantly correlated with temperature, and increases and decreases of *S. bournoni* oocyte abundance followed the temperature regime. These data suggest that the trend of warming or the change in temperature may trigger *S. bournoni* to begin gametogenesis. *Stephanocoenia intersepta* gametogenesis did not show a relationship with increasing temperature indicating that there may be other exogenous factors such as moonlight [26] or day length that cue gamete production. However, similar to other Caribbean corals, maximum mature gamete abundance coincided with the warmest time of the year suggesting that temperature may play a role in gamete maturation [29].

Both species are gonochoric, but three colonies of *S. bournoni* were found to contain individual cosexual polyps that functioned predominantly as male but contained one mature oocyte per polyp. This sexual system is called andromonoecious when male and cosexual polyps are found on the same colony [30]. When male, female, and cosexual polyps are found on separate colonies, this sexual system is called polygamodioecious, and species that have this pattern may be sequential cosexuals with overlap of alternating cycles of male and female function leading to cosexual polyps [30]. However, because such a small proportion of the colonies sampled (3 out of 237 colonies) exhibited this pattern, a small number of polyps (one or two) in the tissue sample displayed this pattern, and only one oocyte occurred in each of the four observed cosexual polyps; it is unlikely that this species is sequential cosexual. Thus, *S. bournoni* can be described as stable gonochoric since it is not unusual to find a low amount of hermaphroditism among gonochoric species [28].

Solenastrea bournoni female fecundity was greater than *S. intersepta* in terms of the number of oocytes per polyp. The number of stage IV oocytes varied between species likely due to differences in oocyte and polyp morphology [31]. The polyps of *S. bournoni* were larger than *S. intersepta*, creating space for a greater number of oocytes. Diameter of *S. intersepta* stage IV oocytes was larger than *S. bournoni* possibly due to a longer period of oogenesis. Fecundity of *S. bournoni* varied between years as has been reported in other species [23] and may be a result of the small number of female colonies sampled prior to spawning. Fecundity *of S. intersepta* was not as variable between years.

The most widely accepted cue for coral spawning is lunar phase [7, 13, 32], and corals have blue-sensitive photoreceptors that have the ability to detect moonlight [33]. Although spawning was not observed directly, histological evidence indicated that *S. bournoni* is a broadcast spawner. No planulae were observed in any of the sampled colonies, and varying stages of gamete development followed by a

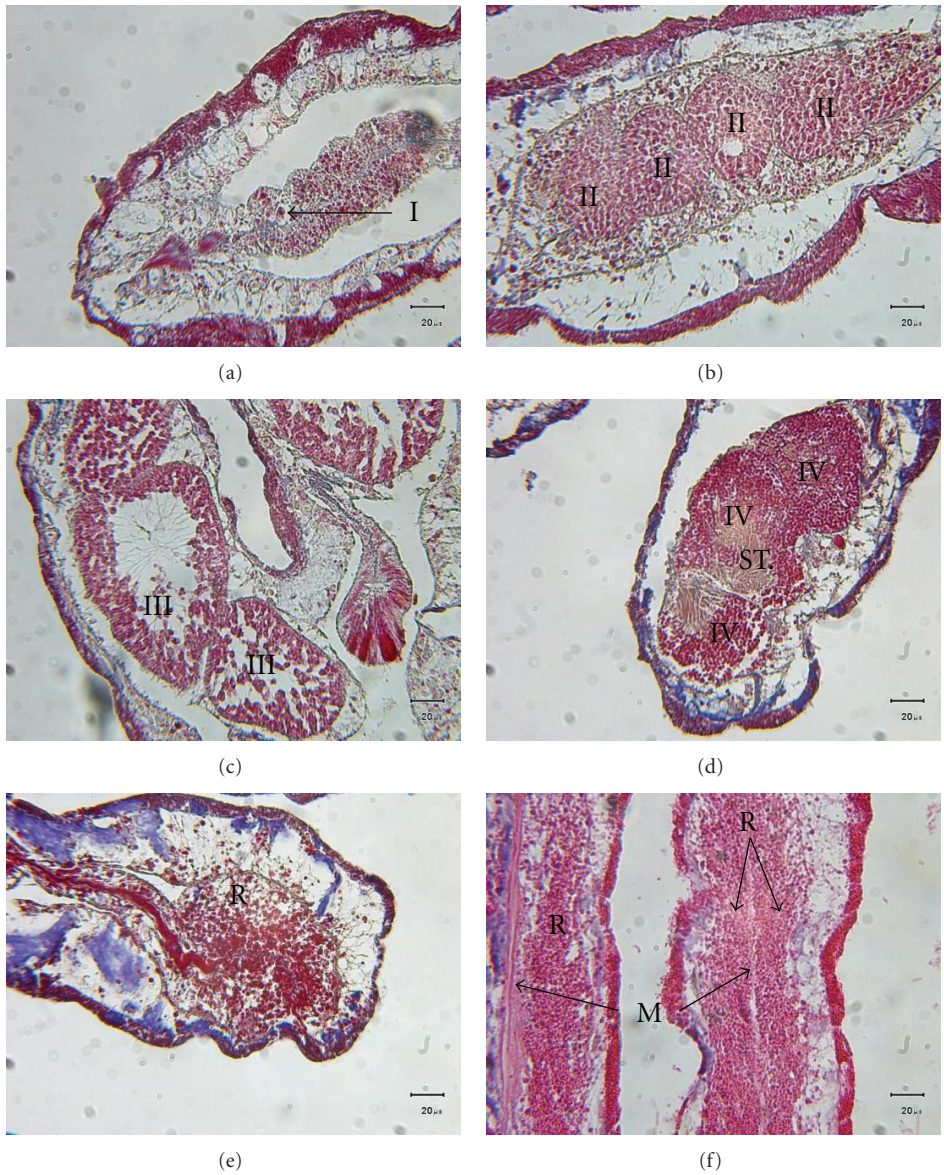

Figure 12: Photomicrographs of *Stephanocoenia intersepta* spermary stages I–IV (defined by Table 1). (a) Stage I spermary; (b) stage II spermaries; (c) stage III spermaries; (d) stage IV spermaries, ST = spermatozoa tails lined up; (e) released spermatozoa (R) stained with Heidenhain's azocarmine aniline blue; (f) released spermatozoa stained with H&E, M = mesoglea. All scale bars 20 μm.

sharp decrease in the abundance of mature gametes indicated spawning activity [24, 34, 35]. Based on results of this study, *S. bournoni* appears to spawn after the full moon of September. Although the main spawning event is thought to occur in September, it is possible that this species may also spawn in August or October due to the presence of mature gametes during these months and the decreases in abundance of mature oocytes. There was a smaller secondary decrease in abundance of mature oocytes after the full moon of October, but small samples sizes before and after the full moon limited interpretation of the data. It is not unusual for a coral species to spawn over more than one lunar period [14–16, 20, 36] especially if gametogenesis is asynchronous [8]. With its short gametogenic cycle and extended periods with mature gametes, it may be possible for *S. bournoni* to spawn over multiple months. Since gametogenesis is closely tied to temperature in this species, spawning may occur sooner or over multiple months in areas closer to the equator. However, we do not have data to confirm these suggestions.

It is likely that *S. intersepta* spawned 5–10 days after the full moon in August or September depending on how early the full moon occurred. Histological evidence suggested that spawning by *S. intersepta* occurred at least 10 days after the 16 August 2008 full moon and at least 5 days after the 4 September 2009 full moon. Spawning could not have been successful if eggs were released after the 6 August 2009 full moon because there were no mature spermaries until late August. Reports from Bonaire indicate that *S. intersepta*

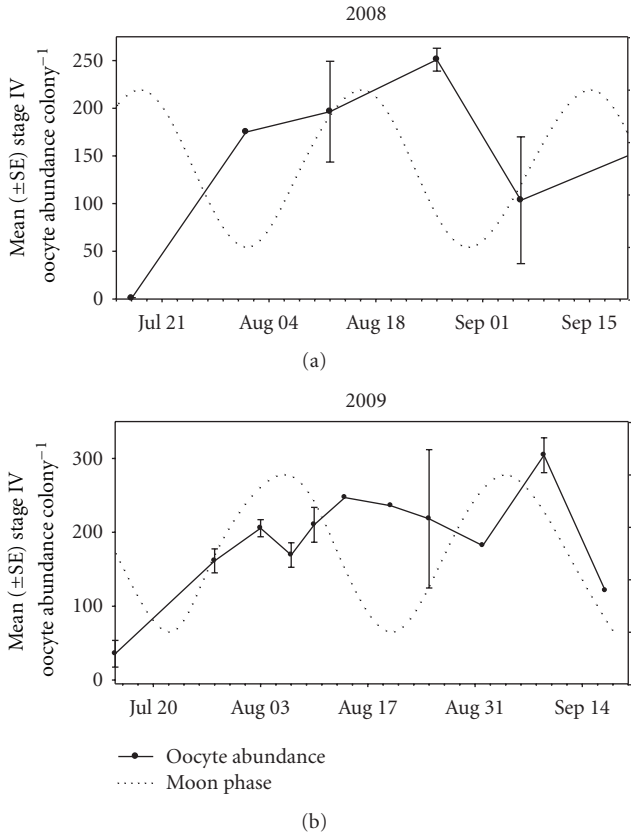

Figure 13: *Stephanocoenia intersepta* mean (±SE) abundance of stage IV oocytes per colony at each sampling date plotted with moon phases for years 2008 and 2009; full moons represented by peaks in moon phase line.

spawns between 3 to 7 nights after the August full moon [16]. At the Flower Garden Banks in the Gulf of Mexico, a region with a difference of only 157 km in latitude from sample sites in southeast Florida, *S. intersepta* spawns 7 to 9 days after the August or September full moon according to Vize et al. [9] and 6 to 10 days after the August full moon as reported by Hagman et al. [20]. Thus, the results of the current study agree with other published records of spawning times from the Caribbean and Gulf of Mexico.

Resorption of oocytes and released spermatozoa may help to confirm spawning times. Resorption of oocytes is not fully understood, but it is thought that by breaking down the large amount of lipid vesicles in oocytes, energy can be absorbed back into the coral [7]. Since oocytes are energetically costly, it would be beneficial to use any excess energy from oocytes that were not released during spawning. Assuming that resorption of oocytes and residual spermatozoa occurs after a spawning event, presence of resorbed gametes may provide additional evidence of when a spawning event occurred. Resorption in *S. bournoni* began in mid-November 2008, indicating that spawning occurred prior to this date. Increased percentages of released spermatozoa in October indicate that spawning may have occurred in September. Resorption and release of spermatozoa in 2009 occurred through much of the year in *S. bournoni*. Oocytes were broken down over a long period of time, and residual spermatozoa that were not viable, as indicated by a lack of purple staining with H&E, persisted for a very long time. High amounts of resorption occurred in November 2009, and high percentages of released spermatozoa were observed in mid-September, again supporting the idea that *S. bournoni* spawned in September. In most coral reproduction studies, samples are not taken year round, and no other examples of this long-term breakdown of gametes were found in the literature. Oocyte resorption over long periods suggests that *S. bournoni* may not immediately require the energy gained from oocyte resorption. Given *S. bournoni*'s delayed onset of gametogenesis until spring, perhaps this extra energy is not needed until production of gametes commences. A slow breakdown may provide energy over a long period of time during winter months when metabolism rates may be slower with cooler temperatures.

Resorption of *S. intersepta* oocytes began in August and occurred in high percentages in September 2008. This observation, coupled with the appearance of a high percentage of released spermatozoa beginning at the end of September, suggests that a spawning event happened before this time and supports the idea of spawning after the August 2008 full moon. All residual oocytes and spermatocytes were gone by early November. In 2009, oocyte resorption was seen in low amounts (less than 2%) from July through early September, but on 17 September, the one female colony sampled showed resorption in 100% of mesenteries. Low percentages of released spermatozoa were observed in September until the 17th when released spermatozoa were at 60%. This evidence supports that spawning of *S. intersepta* occurred between 9 and 17 September 2009. After spawning, *S. intersepta* quickly broke down leftover gametes. *Stephanocoenia intersepta* may need this energy to support gametogenesis which began in December. The role of resorption in coral energetics and its timing relative to gametogenesis needs further study.

In summary, this study provides the first description of the gametogenic cycles of *S. bournoni* and *S. intersepta*. Spawning of *S. bournoni* is thought to occur in southeast Florida between 5 to 14 days after the full moon of September with a possible secondary spawning event occurring after the full moon of October. *Stephanocoenia intersepta* is thought to spawn 2 to 12 days after the late August or early September full moon which is consistent with reports from the Gulf of Mexico and Caribbean. More intense sampling during these potential spawning windows is needed to more precisely pinpoint the exact time of spawning, but this study provides a good foundation for further investigation.

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References

[1] J. R. Guest, A. H. Baird, K. E. Clifton, and A. J. Heyward, "From molecules to moonbeams: spawning synchrony in coral reef organisms," *Invertebrate Reproduction and Development*, vol. 51, no. 3, pp. 145–149, 2009.

[2] M. J. A. Vermeij, E. Sampayo, K. Bröker, and R. P. M. Bak, "The reproductive biology of closely related coral species: gametogenesis in *Madracis* from the southern Caribbean," *Coral Reefs*, vol. 23, no. 2, pp. 206–214, 2004.

[3] A. H. Baird, J. R. Guest, and B. L. Willis, "Systematic and biogeographical patterns in the reproductive biology of scleractinian corals," *Annual Review of Ecology, Evolution, and Systematics*, vol. 40, pp. 551–571, 2009.

[4] E. Weil and W. L. Vargas, "Comparative aspects of sexual reproduction in the caribbean coral genus *Diploria* (Scleractinia: Faviidae)," *Marine Biology*, vol. 157, no. 2, pp. 413–426, 2010.

[5] V. J. Harriott, "Reproductive ecology of four scleratinian species at Lizard Island, Great Barrier Reef," *Coral Reefs*, vol. 2, no. 1, pp. 9–18, 1983.

[6] R. L. Harrison, R. C. Babcock, G. D. Bull, J. K. Oliver, C. C. Wallace, and B. L. Willis, "Mass spawning in tropical reef corals," *Science*, vol. 223, no. 4641, pp. 1186–1189, 1984.

[7] P. L. Harrison and C. C. Wallace, "Reproduction, dispersal and recruitment of scleractinian corals," in *Coral Reefs*, Z. Dubinsky, Ed., pp. 133–207, Elsevier, Amsterdam, The Netherlands, 1990.

[8] S. Brooke and C. M. Young, "Reproductive ecology of a deep-water scleractinian coral, *Oculina varicosa*, from the southeast Florida shelf," *Continental Shelf Research*, vol. 23, no. 9, pp. 847–858, 2003.

[9] P. D. Vize, J. A. Embesi, M. Nickell, D. P. Brown, and D. K. Hagman, "Tight temporal consistency of coral mass spawning at the Flower Garden Banks, Gulf of Mexico, from 1997–2003," *Gulf of Mexico Science*, vol. 23, no. 1, pp. 107–114, 2005.

[10] R. Van Woesik, F. Lacharmoise, and S. Köksal, "Annual cycles of solar insolation predict spawning times of Caribbean corals," *Ecology Letters*, vol. 9, no. 4, pp. 390–398, 2006.

[11] R. C. Babcock, G. D. Bull, P. L. Harrison et al., "Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef," *Marine Biology*, vol. 90, no. 3, pp. 379–394, 1986.

[12] A. Szmant-Froelich, M. Reutter, and L. Riggs, "Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico," *Bulletin of Marine Science*, vol. 37, pp. 880–892, 1985.

[13] Y. H. Fadlallah, "Sexual reproduction, development and larval biology in scleractinian corals—a review," *Coral Reefs*, vol. 2, no. 3, pp. 129–150, 1983.

[14] A. M. Szmant, "Reproductive ecology of Caribbean reef corals," *Coral Reefs*, vol. 5, no. 1, pp. 43–53, 1986.

[15] R. H. Richmond and C. L. Hunter, "Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea," *Marine Ecology Progress Series*, vol. 60, pp. 185–203, 1990.

[16] M. De Graaf, G. J. Geertjes, and J. J. Videler, "Observations on spawning of scleractinian corals and other invertebrates on the reefs of Bonaire (Netherlands Antilles, Caribbean)," *Bulletin of Marine Science*, vol. 64, no. 1, pp. 189–194, 1999.

[17] D. S. Gilliam, "Southeast Florida coral reef evaluation and monitoring project 2010 year 8 final report," Florida DEP Report #RM085, Miami Beach, Fla, USA, 2011.

[18] D. S. Gilliam, R. E. Dodge, R. E. Speiler, C. J. Walton, K. Kilfoyle, and C. Miller, Marine biological monitoring in Broward County, Florida: year 9 Annual Report. Prepared for the Broward County Board of County Commissioners, Broward County Natural Resources Planning and Management Division. pp. 114, 2011.

[19] J. C. Lang, "Status of coral reefs in the western Atlantic: results of initial surveys, Atlantic and Gulf Rapid Reef Assessment (AGRRA) program," Atoll Research Bulletin Technical Report 496, 2003.

[20] D. K. Hagman, S. R. Gittings, and K. J. P. Deslarzes, "Timing, species participation, and environmental factors influencing annual mass spawning at the Flower Garden Banks (northwest Gulf of Mexico)," *Gulf of Mexico Science*, vol. 16, no. 2, pp. 170–179, 1998.

[21] M. J. A. Vermeij, K. L. Barott, A. E. Johnson, and K. L. Marhaver, "Release of eggs from tentacles in a Caribbean coral," *Coral Reefs*, vol. 29, no. 2, pp. 411–411, 2010.

[22] E. A. Chornesky and E. C. Peters, "Sexual reproduction and colony growth in the scleractinian coral *Porites asteroides*," *Biological Bulletin*, vol. 172, pp. 161–177, 1987.

[23] B. Rinkevich and Y. Loya, "Variability in the pattern of sexual reproduction of the coral Stylophora pistillata at Eilat, Red Sea: a long-term study," *Biological Bulletin*, vol. 173, pp. 335–344, 1987.

[24] A. M. Szmant, "Sexual reproduction by the Caribbean reef corals Montastrea annularis and *M. cavernosa*," *Marine Ecology Progress Series*, vol. 74, no. 1, pp. 13–25, 1991.

[25] B. L. Kojis and N. J. Quinn, "1981Aspects of sexual reproduction and larval development in the shallow water hermatypic coral, *Goniastrea australensis* (Edwards and Haime, 1857)," *Bulletin of Marine Science*, vol. 31, pp. 558–573, 1981.

[26] P. L. Jokiel, R. Y. Ito, and P. M. Liu, "Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*," *Marine Biology*, vol. 88, no. 2, pp. 167–174, 1985.

[27] A. Szmant-Froelich, P. Yevich, and M. E. Q. Pilson, "Gametogenesis and early development of the temperate coral *Astrangia danae* (anthozoa: scleractinia)," *Biological Bulletin*, vol. 158, pp. 257–269, 1980.

[28] A. C. Giese and J. S. Pearse, "Introduction: general principles," in *Reproduction of Marine Invertebrates*, A. C. Giese and J. S. Pearse, Eds., pp. 1–49, Academic Press, New York, NY, USA, 1974.

[29] K. Soong, "Sexual reproductive patterns of shallow-water reef corals in Panama," *Bulletin of Marine Science*, vol. 49, no. 3, pp. 832–846, 1991.

[30] J. R. Guest, A. H. Baird, B. P. L. Goh, and L. M. Chou, "Sexual systems in scleractinian corals: an unusual pattern in the reef-building species Diploastrea heliopora," *Coral Reefs*, vol. 31, no. 2, pp. 1–9, 2012.

[31] V. R. Hall and T. P. Hughes, "Reproductive strategies of modular organisms: comparative studies of reef-building corals," *Ecology*, vol. 77, no. 3, pp. 950–963, 1996.

[32] S. C. Wyers, H. S. Barnes, and S. R. Smith, "Spawning of hermatypic corals in Bermuda: a pilot study," *Hydrobiologia*, vol. 216-217, no. 1, pp. 109–116, 1991.

[33] M. Y. Gorbunov and P. G. Falkowski, "Photoreceptors in the cnidarian hosts allow symbiotic corals to sense blue moonlight," *Limnology and Oceanography*, vol. 47, no. 1, pp. 309–315, 2002.

[34] P. W. Glynn, S. B. Colley, C. M. Eakin et al., "Reef coral reproduction in the eastern Pacific: Costa Rica, Panama, and Galapagos Islands (Ecuador) 2. Poritidae," *Marine Biology*, vol. 118, no. 2, pp. 191–208, 1994.

[35] R. G. Waller, P. A. Tyler, and J. D. Gage, "Reproductive ecology of the deep-sea scleractinian coral *Fungiacyathus marenzelleri* (Vaughan, 1906) in the northeast Atlantic Ocean," *Coral Reefs*, vol. 21, no. 4, pp. 325–331, 2002.

[36] B. Vargas-Ángel, S. B. Colley, S. M. Hoke, and J. D. Thomas, "The reproductive seasonality and gametogenic cycle of *Acropora cervicornis* off Broward County, Florida, USA," *Coral Reefs*, vol. 25, no. 1, pp. 110–122, 2006.