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A HISTOLOGICAL ASSESSMENT OF THE REPRODUCTIVE CYCLE OF THE SEA URCHIN, *LYTECHINUS VARIEGATUS*

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Abstract: The regular echinoid *Lytechinus variegatus* is a valuable model for the study of early embryological development. *Lytechinus variegatus* inhabits nearshore seagrass beds in the Gulf of Mexico and Caribbean, and the species ranges from the Carolinas along the US Atlantic coast to Brazil. Evaluating the natural reproductive cycle of *L. variegatus* will aid in understanding its role in community structure and in the management of this resource when housed in the laboratory. From April 2001 to September 2003, at intervals of 4–6 weeks, *L. variegatus* (41–50 mm diameter; n = 32 individuals/collection) were collected at Eagle Harbor in St. Joseph's Bay, FL. A histological staging system for gamete development and maturation of the gonad was devised for female and male *L. variegatus*. The annual minimum gonad index was observed in September or October and increased to a maximum in March or April. A period of nutrient storage and limited gametogenic activity along the germinal epithelium in the fall and winter preceded spring gamete production and maturation. Observations of the mature reproductive stage in *L. variegatus* were associated with the new and full moon, suggesting that lunar cycle or tides influenced the timing of spawning. Peak periods of spawning occurred in spring and, occasionally, in the summer. Spawning probably did not occur from October to January. These data indicate field collections can be timed for early spring to ensure gravid adults for developmental studies.

Key words: echinoid, gonad index, gametogenesis, temperature, staging system

Introduction

The gametes and embryos of the regular echinoid *Lytechinus variegatus* (the variegated sea urchin) are commonly used in genetic and ecotoxicological studies (Zuch and Bradham 2019, Soares and Resgalla 2016), and the adult urchins to supply these gametes are typically harvested from wild populations throughout the year. Understanding the natural reproductive cycle for this species along its range will facilitate improved timing for wild collections to support successful gamete harvest. *Lytechinus variegatus* inhabits nearshore seagrass beds along the Western Atlantic coast from North Carolina in the United States to as far south as Brazil, including the Gulf of Mexico (Watts et al. 2020). Populations of *L. variegatus* spawn throughout the year at the southern end of the range while populations near the northern end of the range have more seasonal spawning, suggesting a latitudinal cline. For instance, near the southern end of the range in Brazil (Junquiera et al. 1997, Garcia and Borzone 2015), Panama (Lessios 1984) and Puerto Rico (Cameron 1986), *L. variegatus* spawned throughout the year; however, in the Brazil and Puerto Rico studies, the gonad cycle showed a trend for larger gonad indices in the summer and fall. Further north in Bermuda and southern Florida, Moore et al. (1963) found that year–round spawning showed peaks in the spring and summer, respectively. In southern Florida, McCarthy and Young (2002) observed spring and fall peaks in induction of spawning. Near the northern end of the range at St. Joseph Bay in the Florida panhandle, Beddingfield and McClintock (2000) observed spring and summer peaks in induction of spawning with KCl.

These and other studies of *L. variegatus* have used changes in gonad indices and induction of spawning to define the reproductive cycle (Moore et al. 1963, Moore and Lopez 1972, Lessios 1991, Beddingfield and McClintock 2000). However, an accurate accounting of the available proportion of gametes in the gonad requires histological examination. Studies in southern Florida (Ernest and Blake 1981, McCarthy and Young 2002) have examined the histology of the ovaries and testes to describe the reproductive cycle of *L. variegatus*. Ernest and Blake (1981) compared the reproductive cycle of *L. variegatus* at 4 sites at Tarpon Springs, FL that varied in water depth, temperature (due to industrial effluent), and availability of food. Histology indicated that habitat differences affected the reproductive cycle of *L. variegatus*; however, general trends of the reproductive cycle were common to all 4 sites, with gonad maturity indicating a major peak of enhanced gamete production in the spring and a minor peak in the summer. At Key Biscayne, FL, McCarthy and Young (2002) observed a major peak in gamete production from April to June and a minor peak in November. In both studies, asynchrony of reproductive stage during the spawning season suggested spawning was asynchronous for the population and occurred as several minor spawning events (Ernest and Blake 1981, McCarthy and Young 2002). After the spring spawning, new oocytes developed on the germinal epithelium while residual ova were absorbed by the nutritive phagocytes (Ernest and Blake 1981). After gametogenesis in the spring, gamete production was continuous until the late summer, when active proliferation of gametes ceased, and synchrony of reproductive stage was re–established during this period of reproductive quiescence (Ernest and Blake 1981).

Previous studies have used various histomorphological criteria to identify stages that are representative of the status of gamete populations in the ovary or testis for numerous regular echinoids (Fuji 1960, Byrne 1990, Walker and Lesser 1998). In this study, we describe a similar staging system for *L. variegatus*. Using this staging system, we evaluated the reproductive cycle during 2001 to 2003 of a population of *L. variegatus* from Eagle Harbor, FL, the northernmost range of *L. variegatus* in the Gulf of Mexico.

Materials and Methods

Field collections and dissections

Lytechinus variegatus were collected at Eagle Harbor in St. Joseph's Bay, FL from April 2001 to September 2003 at intervals of about 4—6 weeks under a Special Activity License SAL—01—0766—SR with Florida Fish and Wildlife Conservation Commission, Division of Marine Fisheries Management. About 32 individuals, ranging in size from 31–55 mm diameter at the ambitus (mean 45.52 mm, median 45.30 mm), were collected during each of the 27 collection times. The habitat at the collection site, about 100 m from shore, was dominated by turtle grass, *Thalassia testudinum,* interspersed with sandy bottoms. The July, August and September collections were made at a site about 100 m farther from shore than the site of other collections due to low tides and resulting mud flats at the Eagle Harbor site.

Water temperature in the bay on the day of collection was recorded to the nearest 1°C, and a water sample in a sealed container was transported to the University of Alabama at Birmingham (UAB) where salinity was measured with a refractometer. Photoperiod was calculated based on the latitude and longitude of Port Saint Joe, FL and the date of collection. Before dissection, the urchins were held for up to 2—3 days in insulated, aerated coolers containing water from the collection site. All housing and procedures on campus were approved by the UAB Institutional Animal Care and Use Committee.

The diameter of individual *L. variegatus* was measured at the ambitus and re—measured at 90° from the first ambital measurement using a caliper. Diameters were determined from the mean of these 2 measurements. The wet urchin was blotted on a paper towel and weighed to the nearest milligram. A circular incision was made in the test between the peristomial membrane and the ambitus. The lantern, gut, and gonad were removed, blotted on paper towels, and weighed separately to the nearest milligram in pre—weighed aluminum pans. Sex was determined from microscopic evaluation of gametes oozing from the gonad during dissection or from a fresh squash of gonad. A sample of fresh gonad was preserved in Bouins fixative for at least 2 days and gonadal tissues were dehydrated, cleared and embedded in paraffin following standard histological techniques. Tissues were sectioned at 7 µm and histological slides were stained with hematoxylin and eosin (H&E). Functionality tests of gametes for fertilization and/or early embryologic development were not conducted in this study. Test, lantern, gut, and gonad were dried to a constant dry weight in a mechanical convection oven at about 60° C for at least 3 days. Wet and dry weight indices were determined for the gonad (GI) using the following formula:

$$GI = (GW/TW)*100 \quad \text{(equation 1)},$$

where GW is gonad weight and TW is total weight of the urchin.

Gonad histology and histomorphometrics

A staging system for gamete development and maturation was devised for female and male *L. variegatus* based on the systems of Walker and Lesser (1998) and Byrne (1990) which consider changes in nutritive phagocytes and gametes during an annual cycle. These staging systems were modified slightly to reflect the overlapping cohorts of gametes of *L. variegatus.* Reproductive stage was determined for individuals based on the predominant condition of acini within an ovary or testis section observed at 100x total magnification with compound light microscopy, and images of each slide were captured with a microscope—mounted digital camera.

The histology of ovaries and testes was divided into 5 stages; spent, partially—spent, renewal, growing and mature. These stages follow the progress of oogenesis and spermatogenesis and the changes in the nutritive phagocyte population that parallel maturation of gametes during the annual cycle of *L. variegatus.*

Oocyte diameters were measured within each ovarian section from digital images for each female *L. variegatus* collected (n = 207 females total). In each of 6 acini, the long diameters of the first 20 ova, parietal oocytes or luminal oocytes with visible germinal vesicles, were measured with the line morphometry function of Optimas 6.2 (n = 120 ova/female). When vitellogenic proteins obscured the germinal vesicle of oocytes, the long diameter was measured if the shape and size suggested a section through a whole rather than a fractional oocyte.

Statistical analyses

Statistical analyses were completed using SAS 9.4. Gonad indices were arcsine transformed and tested for normality using the "PROC UNIVARIATE Normal" procedure. Gonad indices were tested for significant differences between females and males using the "PROC MIXED" procedure on arcsine transformed dry gonad indices with collection date and sex considered as Random effects. Relationships between dry gonad index and temperature or photoperiod were examined using the "PROC REG" procedure. An alpha—level probability <0.05 was considered the threshold for significance.

Results

Gonad index, sea temperature and photoperiod

Values for dry weight gonad indices (n = 207 females and 239 males; Figure 1A) varied annually and seasonally during the 3 years of collections. Female and male dry gonad indices were not significantly different between sexes across the collection times ($F_{1,26} = 0.02$; $p = 0.8957$). Maximum gonad indices were observed in the early spring (Figure 1A), i.e., April of 2001 and 2003 and March 2002 (sea urchins were not collected in April of 2002). Minimum gonad indices for females were observed in September for 2001, 2002, and 2003. For males, minimum gonad indices were observed in August, October, and September for 2001, 2002, and 2003, respectively.

Dry gonad indices were significantly correlated with temperature for both sexes (females $F_{1, 205} = 78.95$; $p < 0.001$; males $F_{1, 237} = 69.62$; $p < 0.001$). Increasing sea temperatures were associated with decreasing dry gonad indices for females and males (Figure 2A, B). The annual variation in range of gonad indices may be related to the magnitude of winter water temperatures. The highest gonad indices for females and males (Figure 1A) of this 3—year study were observed in 2002 following a relatively mild winter (sea temperature ranged from 14 to 23°C, Figure

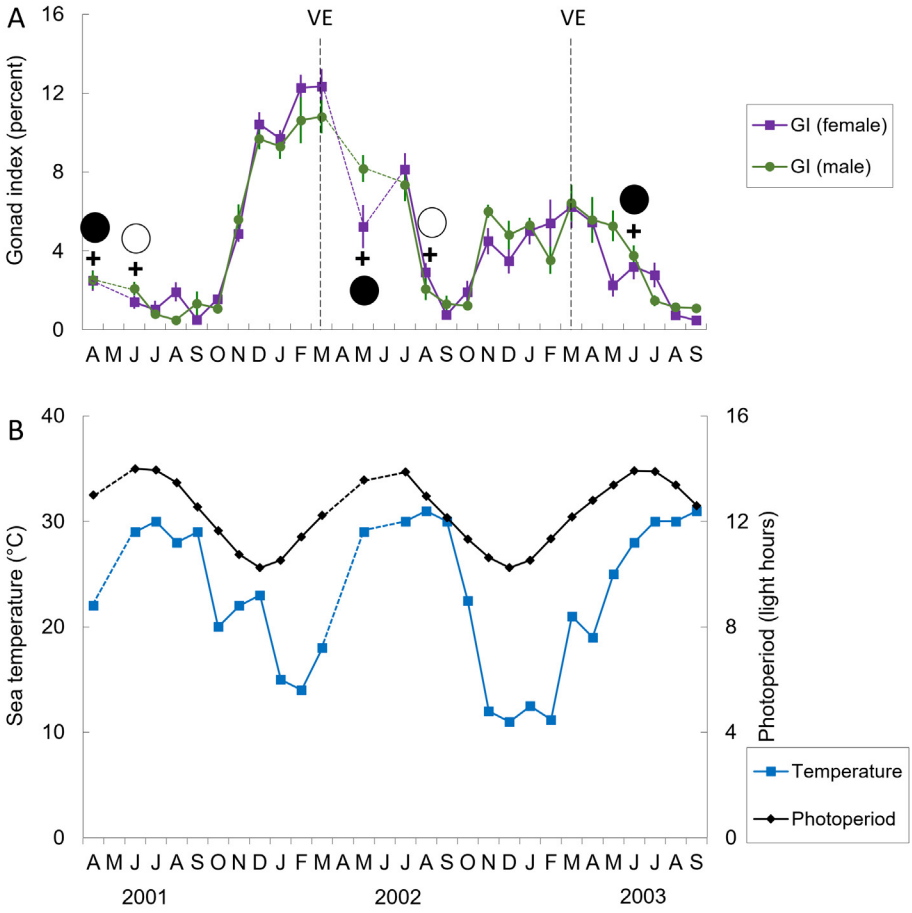

FIGURE 1. *Gonadal indices and environmental data collected between April 2001 and September 2003 from Eagle Harbor, St. Joseph Bay, FL. A. Mean (± SE) dry gonad indices for female and male* Lytechinus variegatus, *n = 4–13 per sex per collection. Crosses indicate collections that included at least one mature stage gonad of either sex. Black and white circles represent new and full moons, respectively. Dashed vertical lines represent occurrence of the vernal equinox. B. Sea temperature and photoperiod at time of collection. Dotted lines occur during months when collections were not made.*

1B). Gonad indices were lower for both females and males following a relatively cold winter in 2003 (sea temperature ranged from 11 to 12.5°C). Gonad indices increased sharply from October to December in both 2001 and to a lesser extent in 2002 (Figure 1A). In 2002 and 2003 observed maxima for gonad indices appeared within about a month of spring increases in sea temperature; no data prior to the spring peak in 2001 were available. The sea temperatures observed at spring maxima of gonad indices were 22, 18, and 19 °C for 2001, 2002, and 2003, respectively (Figure 1B). Minor summer peaks in gonad indices were observed only in females and did not show a consistent relationship with temperature.

Dry gonad indices were also correlated with photoperiod (females $F_{1, 205} = 34.24$; $p < 0.001$; males $F_{1, 237} = 48.43$; $p < 0.001$). Increasing day length was associated with decreased dry gonad indices for females and males (Figure 2C,D). Annual gonad indices reached the minimum within 4 weeks following the autumnal equinox and increased to the major peak in March and April, within 4 weeks of the vernal equinox (Figure 1A). The decrease in gonad index from the spring peak was associated with the increase in photoperiod until the summer solstice in late June. Consequently, the second, minor peak in gonad index observed only in females in August 2001, July 2002, and June 2003 occurred within one or 2 months of the summer solstice and the beginning of decreasing photoperiod. In general, the major spring increase in gonad indices occurred as photoperiod was increasing. The summer, minor peak in gonad index varied in its proximity to the summer solstice and decreasing photoperiod.

Histology and reproductive stages of the gonads

Spent stage

In spent stage ovaries (Figure 3A), the central lumen of acini remained unstained with H&E or contained some residual ova stained a deep pink with H&E. Developing oocytes were sometimes sparsely distributed along the germinal epithelium. In some spent acini, especially those collected in summer months, irregular–shaped brown granules were present in the lumen. The lumen of spent acini sometimes contained many partial remnants of phagocytized oocytes. These were small, circular, and often had a visible nucleolus and a narrow band of vitellogenins. Nutritive phagocytes formed a thin border along the acinus lumen or formed a sparsely–stained network within the lumen. Other absorbing residual material present in the lumen stained intensely purple in H&E and may have been residual nuclear material. In testes (Figure 3B), the central lumen of acini remained unstained with H&E or contained residual sperm. Nutritive phagocytes formed a thin border along the acinus lumen or a sparsely–stained network within the lumen. Irregular, purple granules were phagocytized sperm.

Partially-spent stage

In partially–spent stage ovaries (Figure 3C), some moderately large unstained areas were present in the acinus, especially in the central lumen. The acinus lumen contained many vitellogenic oocytes with pre–vitellogenic oocytes localized to the germinal epithelium. In some cases, small, oval oocytes were clumped together in several masses within the lumen. In other instances, the oocytes were indistinguishable from other pre–vitellogenic oocytes of growing stage. Nutritive phagocytes were absent or formed a sparsely–stained network of cells near the oocytes. In partially–spent testes (Figure 3D), some moderately large areas of the central lumen were unstained. The central lumen often contained residual sperm. Many residual spermatocytes were present along the germinal epithelium, forming

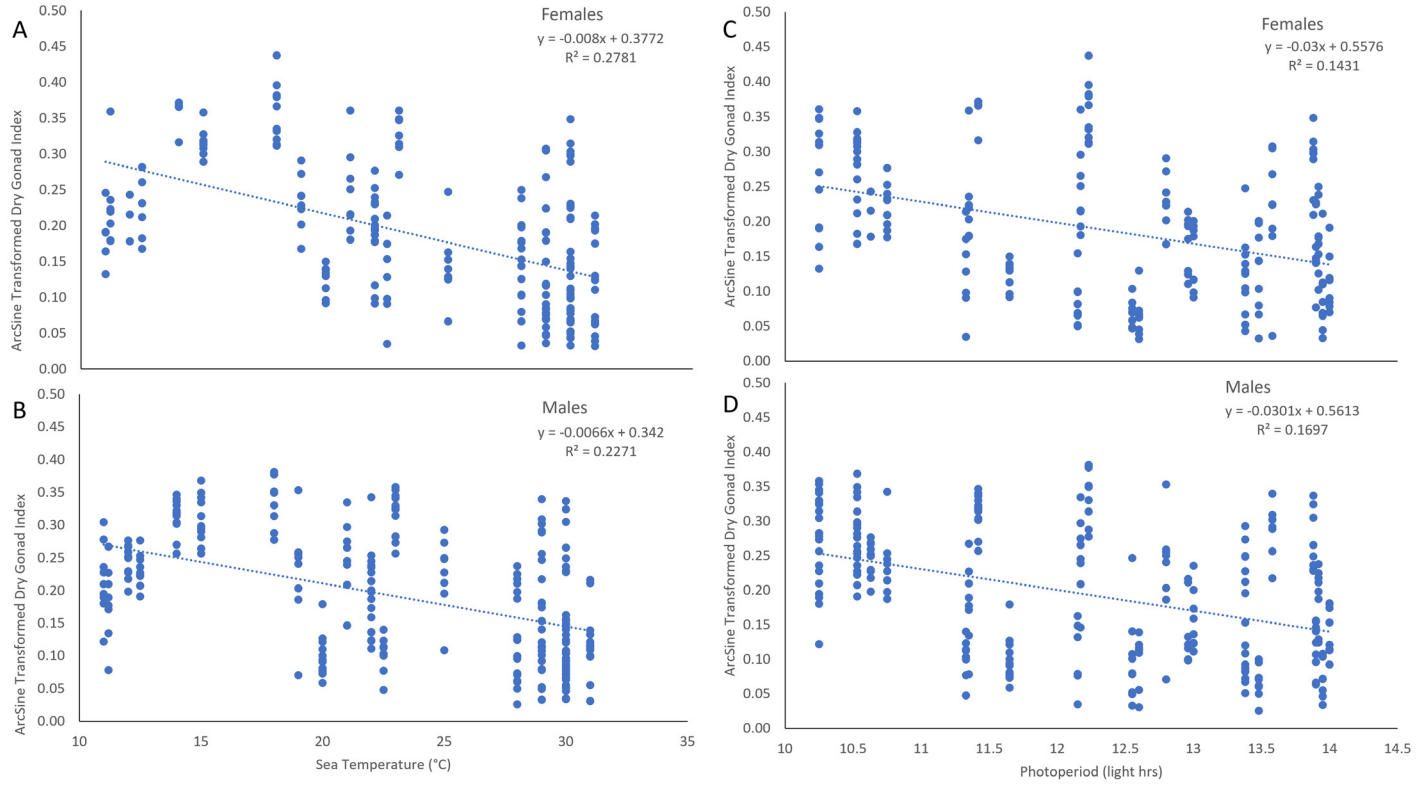

FIGURE 2. *Correlation of dry gonad index of* Lytechinus variegatus *with environmental variables collected between April 2001 and September 2003 from Eagle Harbor, St. Joseph Bay, FL. A. Females with temperature. B. Males with temperature. C. Females with photoperiod. D. Males with photoperiod. n = 4 to 13 individuals per sex at each collection; total of 207 females and 239 males. Values represent arcsine transformed dry gonad indices.*

irregular, disorganized columns generally detached from the acinus wall.

Renewal stage

In renewal stage ovaries and testes (Figure 4A, B), intensively–stained nutritive phagocytes comprise most of the lumen of the acini. In ovaries (Figure 4A), some primary oocytes were beginning to develop on the germinal epithelium. Small numbers of residual oocytes or ova were sometimes present in the central lumen. In testes (Figure 4B), developing spermatogonia and spermatocytes sometimes formed a thin layer, usually sparsely distributed in small patches along the germinal epithelium. Small, purple residual material, possibly phagocytosed gametic material, was present in both sexes.

Growing stage

In growing stage ovaries (Figure 4C), parietal (attached), pre–vitellogenic oocytes were developing on the germinal epithelium. Parietal oocytes were oriented parallel to the acinus wall and then, as they matured, changed orientation to perpendicular. Germinal vesicles were sometimes visible in the parietal oocytes. Vitellogenic oocytes had detached from the germinal epithelium and were located in the lumen. These luminal oocytes usually had a visible germinal vesicle and nucleolus. A few mature ova were sometimes present in the lumen. Mature ova stained a lighter shade of pink than oocytes along

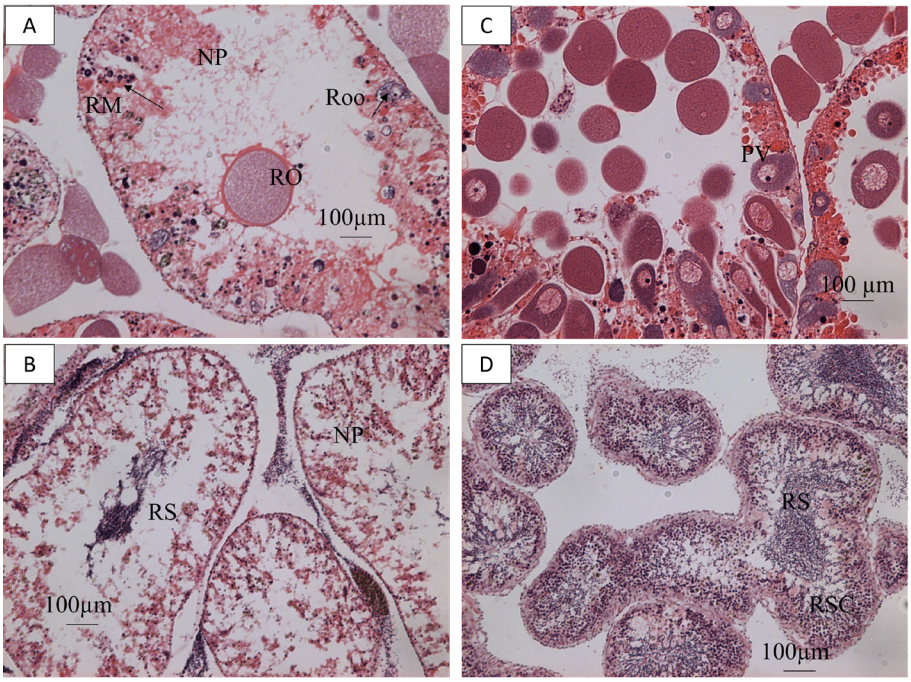

FIGURE 3. *Histological sections of ovaries and testis of* Lytechinus variegatus. *A. Ovaries in spent stage. B. Testes in spent stage. C. Ovaries in partially–spent stage. D. Testes in partially–spent stage. NP–nutritive phagocytes; PV–previtellogenic oocytes; RM–residual material; RO–residual ova; Roo–residual oocytes; RS–residual sperm; RSC–residual spermatocytes.*

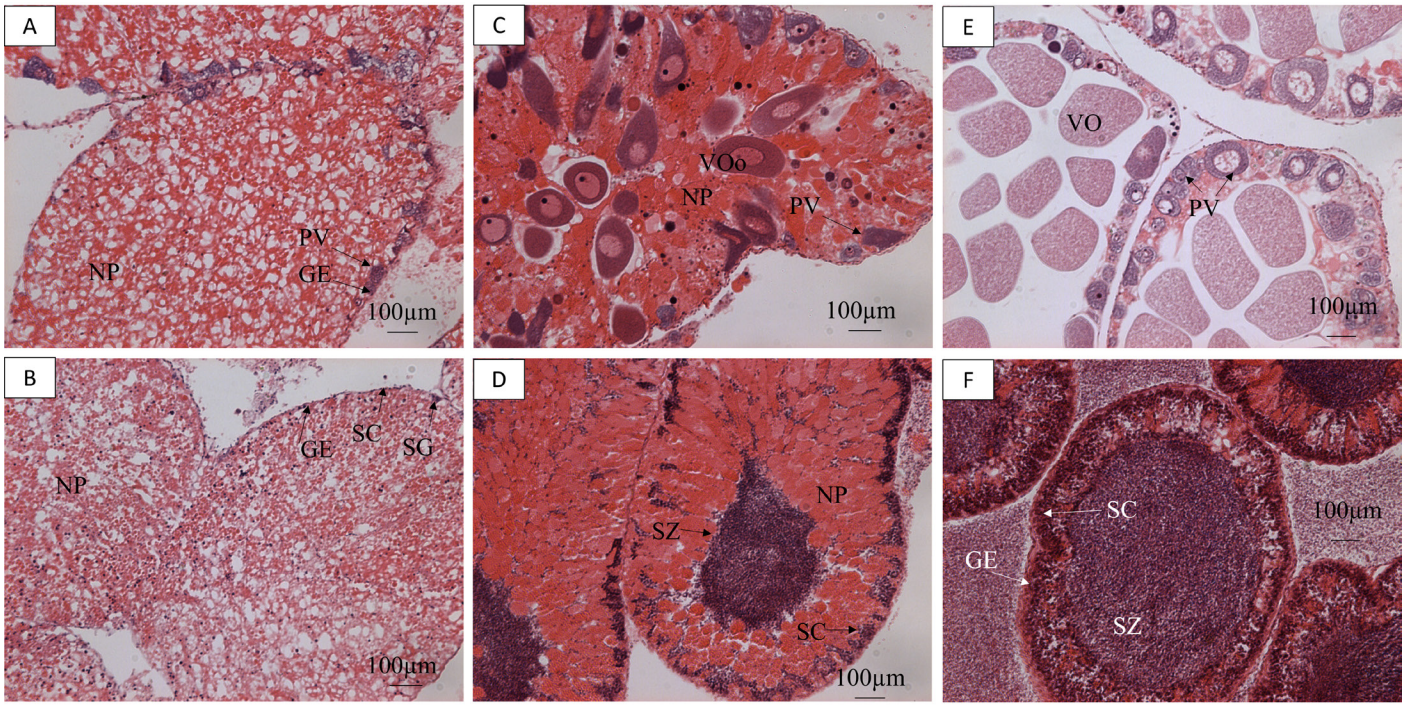

FIGURE 4. *Histological sections of ovaries and testis of* Lytechinus variegatus. *A. Ovaries in renewal stage. B. Testes in renewal stage. C. Ovaries in growing stage. D. Testes in growing stage. E. Ovaries in mature stage. F. Testes in mature stage. GE–germinal epithelium; NP–nutritive phagocytes; PV–pre–vitellogenic oocytes; SC–spermatocytes; SG–spermatogonia; SZ–spermatozoa; VOo–vitellogenic oocytes; VO–vitellogenic ova.*

the germinal epithelium, which would gradually stain lighter shades of pink as they underwent meiotic divisions and were observed in the lumen. Nutritive phagocytes formed a densely–stained pink network within the lumen and extending from the wall of the acinus. In testes (Figure 4D), spermatocytes on the germinal epithelium formed columns extending into the lumen and some spermatozoa had accumulated in the lumen, stained purple. Nutritive phagocytes formed a densely–stained pink network within the lumen.

Mature stage

In mature stage ovaries (Figure 4E), eosinophilic, vitellogenic ova filled the central lumen. Nutritive phagocytes were absent or formed a fine mesh layer in proximity to the germinal epithelium. Previtellogenic oocytes in various stages of development were in this layer. In testes (Figure 4F), mature spermatozoa filled most of the lumen. Nutritive phagocytes formed a thin layer in proximity to the germinal epithelium. Scattered spermatocytes in this layer were in sparse columns or appeared disorganized.

Distribution of reproductive stages

In general, most of the ovaries and testes from October to February were in the renewal stage (Table 1). All males and females were in the renewal stage in November and December 2001 and 2002 and January 2002 and 2003 with the exception of November 2001 females and January 2003 males, where some sea urchins were in the growing stage (10% and 11%, respectively, Table 1). All males and females were in the growing stage in March 2002 and March and April 2003; sea urchins were not collected in March 2001 (Table 1). For all other months, 2 or more stages were observed. In these cases, ≥50% of the specimens captured each month were in a single stage with the exception of females in April 2001 and September 2003 and males in September 2002.

Over the 3 years of this study, collections showed considerable variation regarding the earliest and latest observations of the spent reproductive stage. The spent stage was first observed in females in June 2001 and July 2002, but not until September in 2003 (Table 1). In 2002, the June collection was not analyzed because the collected urchins spawned in transit from St. Joseph Bay. The spent stage was first observed in males in June 2001, September 2002 and February 2003 (Table 1). The latest observations of the spent stage were in September for females in all 3 years. For males, the latest observations of the spent stage were in October 2001 and 2002; sea urchins were not collected in October of 2003, but spent males were seen in September 2003.

Less than 2% of all sea urchins collected were in the mature stage (Table 1). Mature females were only found in April 2001, May and August 2002 and June 2003. Mature males were observed in June 2001 and August 2002. All mature stages (cross symbols in Figure 1, 6 females and 3 males) were observed in sea urchins collected on the day of the new moon or within 3 days of the new or full moon and were not observed at any other phase of the moon.

Oocyte and ova diameters

Ovaries of individuals from all collections in 2001, 2002, and 2003 included some small oocytes with diameters measuring 5–70 µm (Figure 5). From February through Septem-

TABLE 1. *Distribution of reproductive stages of female (F) and male (M) Lytechinus variegatus collected between April 2001 and September 2003 from Eagle Harbor, St. Joseph Bay, FL. Values represent percentages of each sex in each reproductive stage. N – number of samples collected each month.*

Year	Month	N		Spent		Partially-spent		Renewal		Growing		Mature	
		F	M	F	M	F	M	F	M	F	M	F	M
2001	April	7	9	0	0	14	78	29	0	29	22	29	0
	May	–	–	–	–	–	–	–	–	–	–	–	–
	June	9	8	33	38	11	0	56	38	0	0	0	25
	July	9	8	44	33	11	0	44	67	0	0	0	0
	August	9	9	0	60	22	30	0	10	78	0	0	0
	September	8	9	63	44	38	22	0	22	0	11	0	0
	October	8	12	0	8	0	0	100	92	0	0	0	0
	November	10	9	0	0	0	0	90	100	10	0	0	0
	December	8	12	0	0	0	0	100	100	0	0	0	0
2002	January	8	11	0	0	0	0	100	100	0	0	0	0
	February	5	12	0	0	0	0	60	31	40	69	0	0
	March	10	8	0	0	0	0	0	0	100	100	0	0
	April	–	–	–	–	–	–	–	–	–	–	–	–
	May	8	8	0	0	0	0	63	100	25	0	13	0
	June	–	–	–	–	–	–	–	–	–	–	–	–
	July	8	8	10	0	0	13	10	25	80	63	0	0
	August	8	8	0	0	50	50	0	13	25	25	25	13
	September	10	6	60	33	40	33	0	17	0	17	0	0
	October	7	8	0	33	14	0	86	67	0	0	0	0
	November	3	10	0	0	0	0	100	100	0	0	0	0
	December	5	9	0	0	0	0	100	100	0	0	0	0
2003	January	6	9	0	0	0	0	100	89	0	11	0	0
	February	7	8	0	25	0	0	100	75	0	0	0	0
	March	8	8	0	0	0	0	0	0	100	100	0	0
	April	8	8	0	0	0	0	0	0	100	100	0	0
	May	8	8	0	0	25	13	25	0	50	87	0	0
	June	8	8	0	0	13	0	50	88	25	13	13	0
	July	7	9	0	0	0	0	43	56	57	44	0	0
	August	6	9	0	0	33	25	67	75	0	0	0	0
	September	9	8	33	13	33	13	33	75	0	0	0	0

No collections were assessed in May 2001, April 2002, or June 2002, signified by the dash (–).

ber, larger oocytes (≥100 µm in diameter) were always present (Figure 5B, C, D). Spring and summer months included a small percentage of ova ≥ 150 µm (Figure 5C, D). In the spring, increased frequency of oocytes > 150 µm in diameter were observed in April 2001 and 2003 and in March 2002 and 2003 (sea urchins were not collected in April 2002). A similar increase in larger oocytes in the summer were observed in August 2002 and in July 2003.

Discussion

General Characteristics of the Reproductive Cycle

Data from the present study indicate the reproductive cycle of *L. variegatus* at Eagle Harbor is a biannual cycle, an unusual cycle for regular echinoids, but one that has been observed in gonad histology of *L. variegatus* in warmer waters at Tarpon Springs, FL (Ernest and Blake 1981) and at Key Biscayne, FL (McCarthy and Young 2002). To our knowledge, the present study is the northernmost to examine histology of the reproductive cycle of *L. variegatus*. For *L. variegatus* at Eagle Harbor, the season of gamete production and spawning generally extends from about March to October. We found that peak periods of spawning occurred in the spring and, inconsistently, in the summer. The general patterns of nutrient storage and gamete production observed in this study were consistent with those observed in other temperate sea urchin species (*Strongylocentrotus nudus, S. intermedius*; Fuji 1960; *Paracentrotus lividus*, Byrne 1990; *Pseudechinus* sp., McClary and Barker 1998; *Evechinus chloroticus*, Brewin et al. 2000).

Stages of reproduction and the reproductive cycle

Because sea urchin gonads are dual–function organs (i.e., nutrient storage and gamete production), changes in gonad size and therefore gonad index may reflect changes in nutrient

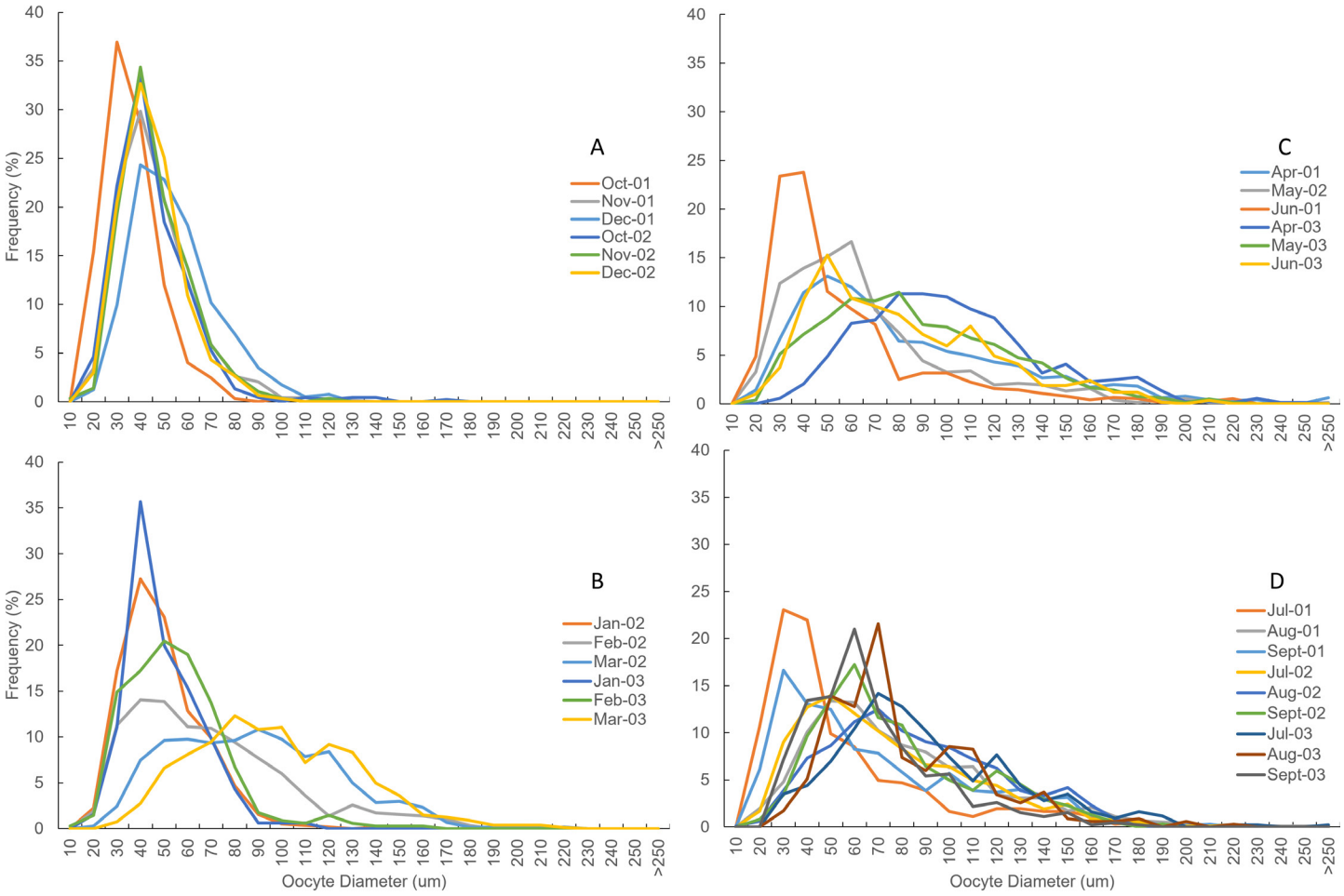

FIGURE 5. *Oocyte diameter frequency of* Lytechinus variegatus *collected between April 2001 and September 2003 from Eagle Harbor, St. Joseph Bay, FL. A. Fall and early winter 2001 and 2002. B. Late winter and early spring 2002 and 2003. C. Late spring and early summer 2001–2003. D. Mid– to late summer 2001–2003. N = 4 to 8 females per collection.*

storage, total volume of gametes, or both. To describe gonadal changes in the gametic and somatic cellular populations during the gonad cycle, a staging system was devised for *L. variegatus* based on the Byrne (1990) system for *Paracentrotus lividus*, and incorporates 5 stages: spent, partially–spent, renewal, growing, and mature. However, the staging system devised for *L. variegatus* differs in several important ways from the stages devised by Byrne (1990). For instance, the partially–spent stage for this study is used only to indicate a gonad containing acini with many immature gametes, low levels of nutritive phagocytes, and extensive unstained areas, suggesting a minor or partial spawning event had occurred recently. The premature stage of Byrne (1990) is not included in our description of *L. variegatus* because ovaries and testes did not pass through a distinct stage during which gametes were clustered near the germinal epithelium. Instead, more advanced gametes were observed more in the central lumen of acini while new gametes formed on the germinal epithelium. In mature ovaries, dissected gonads were usually very soft and oozed many gametes. Acini from these ovaries were not densely packed with ova when observed in histological sections but were designated "mature" if many mature ova were present in the lumen of the acini and oocytes were generally confined along a narrow strip of nutritive phagocytes near the germinal epithelium.

We consider the spent stage to be a relatively transient stage based on the low number of observations (n = 22 females, 23 males). In the spent reproductive stage, nutritive phagocytes are highly vacuolated and poorly stained with eosin, suggesting that nutritive reserves are largely depleted. Rapid restoration of the nutritive phagocytes enables sea urchins to begin storing nutrients for future gamete production or for use during periods of nutrient deprivation. Gamete production may be delayed until nutrients in the gonad have reached a certain level (Pearse 1969). Therefore, there would be strong selective pressures to restore nutritive phagocytes as quickly as possible after spawning.

The partially–spent stage was often associated with periods of high or low temperatures. Most partially–spent gonads were observed after the unusually low winter temperatures of 2000 and during the summer months when temperatures were at annual maxima. In a partially–spent gonad, the presence of empty lumen within an acinus and many immature gametes

along the germinal epithelium suggested that relatively few ova or sperm reached maturity before spawning. Possible explanations include that the partially—spent stage is an alternate strategy of gamete production and spawning, allowing sea urchins to release at least some gametes in response to spawning cues. Alternatively, nutrient transfer to gametes may not have been adequate for maturation of an entire cohort, or the viability of developing gametes may have been compromised. Because the partially—spent stage was frequently observed in the summer, it is possible that summer spawning events may involve fewer gametes than spring spawning events when the spent stage was more commonly observed. Large numbers of immature oocytes, indicated by vitellogenic oocytes with diameter < 100 μm, remaining within the lumen during the partially—spent stage may either continue to develop or be reabsorbed. If immature oocytes develop, they may pass through a quiescent period while nutritive phagocytes are replenished or they may receive nutrients directly from the coelomic fluid to continue maturation (Unuma et al. 2010). However, it seems more likely that these remaining oocytes and ova do not develop further and are absorbed, supported by observations of the relatively large numbers of absorbing oocytes observed in summer. In the summer, ova and oocytes may be absorbed because *L. variegatus* has less capacity to supply them with nutrients. Summer temperatures are negatively correlated with gonad production and gamete development, and impact nutrient absorption from the diet (Gibbs and Watts 2004, Gibbs et al. 2007 Watts et al. 2011).

Although gamete development and spawning occurred during the spring and summer, individual sea urchins that were not actively producing gametes were also observed during these months. These sea urchins that are primarily storing nutrients (in renewal stage) may be urchins that have not yet spawned during the current reproductive season or they may be accumulating nutrients in preparation for their next cohort of gametes. For much of the reproductive season, *L. variegatus* was observed either in a state of primarily accumulating nutrients (renewal stage) or in the early stages of gamete development (growing stage). Possibly, these stages require more time and gamete maturation and spawning are relatively rapid.

Unlike other sea urchin species with a distinct growing stage characterized by parietal oocytes and a premature stage characterized by luminal oocytes (Byrne 1990, Walker and Lesser 1998, McClary and Barker 1998, Brewin at al. 2000), *L. variegatus* begin to develop new oocytes along the germinal epithelium while more mature oocytes accumulate yolk proteins and are localized in the lumen. *Lytechinus variegatus* produces overlapping cohorts of gametes, a strategy for rapid production of gametes consistent with ruderal species (Lawrence and Bazhin 1998). Based on the low number of observations of mature stage urchins, it appears that the mature stage is relatively transient. Apparently, mature ova of *L. variegatus* are seen in the lumen for a relatively brief period except when they are not released at spawning and are absorbed. Once echinoid gonads fill with gametes, they are sensitive to a variety of cues, including mechanical stimulation, and spawning probably occurs within a few days (Lessios 1991), as seen in this study by urchins spawning during transport to the laboratory in June 2002.

Observations of ova diameters provide supporting evidence that ova develop in overlapping cohorts and new cohorts of gametes are continuously developing on the germinal epithelium. When larger oocytes and ova are present, a wide range of diameters of oocytes are usually found. Size—frequency shifts to smaller sizes of oocytes indicate that spawning occurs during various months throughout the spring and summer. By October, most spawning has been completed for the annual cycle and many *L. variegatus* have begun to store nutrients in preparation for the next season of gamete production. From about November through February, nutrient storage is the primary function of the gonad. During these months, small oocytes on the germinal epithelium may be dormant or they may grow slowly. The time for maturation of a single ovum or of a single cohort of ova is difficult to determine from observations of oocyte and ova diameters because mature eggs can be retained until they are absorbed, and their nutrients are recycled for the next cohort of eggs.

Synchrony of reproductive stage can be observed in the winter and early spring gonads, when most individuals are in the renewal stage. Later in the spring, after some spawning is likely to have occurred, asynchrony of stages among individuals may be caused by individual differences in the rates of nutrient accumulation, gametogenesis, and innate differences in sensitivity to exogenous cues. Similar periods of asynchrony of reproductive stage during the spawning season followed by synchrony during periods of renewal have been observed for *L. variegatus* populations on the Atlantic and Gulf of Mexico coasts of Florida (Ernest and Blake 1981, McCarthy and Young 2002). Foraging patterns that provide more nutrients, especially protein (Hammer et al. 2006), may promote gamete production and contribute to the asynchrony of reproductive stages observed in the spring and summer. Diet quantity and quality affect the time required for gamete production (Dix 1970, Walker and Lesser 1998, Beddingfield and McClintock 2000, Hammer et al. 2006).

Exogenous factors affecting the reproductive cycle

Within the range of temperatures observed during the 3 years of this study, temperature extremes were inversely related to gonad size. During warmer months, gonads were typically found in the spent or partially spent stages and colder months were often associated with renewal and growing stages. In the laboratory, sea urchins maintained at 16 or 28°C had lower wet gonad weights than sea urchins held at 22°C, which were attributed in part to differences in feed intake, nutrient processing, and metabolic demand at these temperatures (Watts et al. 2011). Gibbs et al. (2007) found that gamete production of lab—cultured *L. variegatus* was significantly reduced at 28°C, a temperature which is typical during the summer at Eagle Harbor, suggesting summer temperatures may limit gamete production.

Mild temperatures in the fall and winter of 2001 were associated with increased nutrient storage and gamete produc-

tion in *L. variegatus* at Eagle Harbor, resulting in an increase in the gonad index. The mild fall and winter temperatures in 2001 may have increased abundance of some food organisms for *L. variegatus* or may have optimized production in the gonad through metabolic effects on nutrient processing (Watts et al. 2011, Gibbs et al. 2007). Mild temperatures, because of their effect on nutrient storage, could be an indirect cue for early gametogenesis. The increase in percentage of *L. variegatus* in the growing stage during the spring of 2002, following the mild 2001 winter, may have occurred because gametogenesis occurs as soon as nutrients stored in the gonad reach a certain level (Pearse 1969). *Lytechinus variegatus* process proteins and lipids from the diet more efficiently at 16 and 22°C (Gibbs et al. 2007), which are temperatures similar to the mild fall and winter 2001 temperatures observed in this study. Improved utilization of proteins and lipids from the diet may better support oogenesis and nutrient provisioning in oocytes.

Temperatures in the shallow St. Joseph Bay may increase as eroding sediment from surrounding areas enters the bay and decreases depths. More research is needed to understand the effect of rising temperatures on the population of *L. variegatus* of St. Joseph Bay. Climate change forecasts higher sea temperatures, lower pH, and higher prevalence of carbon dioxide in ocean environments (reviewed by Gattuso et al. 2018), all of which may impact food availability for and physiologic function of sea urchins. Studies in the Australian echinoid, *Heliocidaris erytrogramma*, revealed that elevated housing temperatures of 4°C above ambient sea temperatures, indicative of near—future climate change scenario for the region, reduced successful embryonic development by 40 — 90% (Byrne et al. 2009). Ocean—warming simulation in *L. variegatus* found that larval development at 31°C was faster and produced smaller larvae than larvae at 28°C (Lenz et al. 2019). The combined effects of temperature on feed intake, nutrient processing, nutrient allocation for growth, gametogenesis, and embryonic/larval development (Watts et al. 2011, Gibbs and Watts 2004, Gibbs et al. 2007, Lenz et al. 2019) suggest near—future increases in ocean temperature may impact the persistence of near—shore populations of *L. variegatus*. The data presented here represent the state of the reproductive cycle in *L. variegatus* at Eagle Harbor during the early years of the 21st century, and these data can provide valuable context to which comparisons can be made for the current population.

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