2007

Effect of Temperature on Gamete Production and Biochemical Composition of Gonads in the Sea Urchin *Lytechinus variegatus*

Victoria K. Gibbs
University of Alabama at Birmingham

Stephen A. Watts
University of Alabama at Birmingham

Addison L. Lawrence
Texas A&M Agricultural Experiment Station

Effect of Temperature on Gamete Production and Biochemical Composition of Gonads in the Sea Urchin *Lytechinus variegatus*

VICTORIA K. GIBBS, STEPHEN A. WATTS, AND ADDISON L. LAWRENCE

Temperature is one of the most important proximate factors affecting the biology of ectothermal organisms. In the sea urchin, *Lytechinus variegatus*, the reproductive cycle in wild populations is correlated with changing water temperature, suggesting that reproduction may be dependent, in part, on temperature. Adult *L. variegatus* (ca. 35.63 ± 1.24 g wet weight, 40-mm diameter) were collected in October 2001 from St. Joseph Bay, FL (30°N, 85.5°W) and transported to the University of Alabama at Birmingham. Sea urchins were placed into nine 80-liter aquaria (n = eight sea urchins per aquarium) maintained in enclosed incubators (n = three aquaria per incubator) at a specific constant temperature of 16, 22, or 28°C and 32 ppt salinity synthetic seawater (Instant Ocean). Within each aquarium, individuals were maintained in 1-liter containers with recirculation and were fed daily a formulated feed ad libitum for 8 wk. At the end of week 8, final measurements of each individual were recorded, individuals were dissected, gonads were measured, and gonad histology and biochemistry were analyzed. Gonad weights were highest for individuals held at the 22°C treatment, but did not vary between individuals held at 16 or 28°C. The acinus volume in the gonad was occupied primarily by nutritive phagocytes at all temperature treatments. In females, gamete volumes were highest for females held at 22°C, whereas gamete volumes were not different for females held at 16 or 28°C. In males, gamete volumes were significantly lower at 28°C, and gamete volumes were not different between males held at 16 or 22°C. Gamete volumes were small in all temperature treatments, suggesting that gamete production had not substantially advanced within the 8-wk study period. The cellular ultrastructure of the nutritive phagocytes varied with temperature. Vacuolated nutritive phagocytes were common in the acini of individuals held at 16°C, and globulated nutritive phagocytes were common in the acini of individuals held at 28°C. Females held at 22°C had the highest protein content in the gonad, and protein content was not different between females held at 16 or 28°C. The amount of lipid was highest for males held at 16°C and did not differ between males held at 22 or 28°C. These data lead us to suggest that *L. variegatus* utilize different nutrient allocation strategies in the gonad in response to temperature, which could affect the reproductive success of the species if subjected to long-term changes in seawater temperature.

L*ytechinus variegatus* is a temperate to tropical species of sea urchin found along the Atlantic coast from North Carolina to the Florida Keys, in the Gulf of Mexico, through the Caribbean, and as far south as southern Brazil (Moore et al., 1963). Within the higher latitudes, *L. variegatus* are subject to a wide range of temperatures. In the seagrass beds of St. Joseph Bay on the Florida Gulf Coast, *L. variegatus* can experience temperatures as low as 11°C in winter months and as high as 36°C in summer months (Beddingfield and McClintock, 2000). Temperature affects several parameters in *L. variegatus*, including growth, consumption, feeding rate, digestion and absorption, and metabolic rate (Moore et al., 1963; Moore and McPherson, 1965; Ernest and Blake, 1981; Klinger et al., 1986; Hofer, 2002). The direct effect of temper-

ature on gametogenic cycles and possible changes in gonad biochemistry has not been determined for this species.

The gonad of *L. variegatus*, like most sea urchins, is composed of five lobes located beneath the interambulacral plates of the test. Each gonad lobe contains hundreds of tubules known as acini that often give the gonad an appearance of a cluster of grapes (Unuma, 2002). The parietal layer of the acinus is commonly referred to as the germinal epithelium and is comprised of two primary cell types: germ cells (ova or sperm) and somatic cells known as nutritive phagocytes (Pearse and Cameron, 1991). Nutritive phagocytes store nutrients that can be mobilized to developing germ cells or used to sustain physiological processes during periods of starvation (Holland

and Giese, 1965; Spirlet et al., 2000; Walker et al., 2001; Unuma, 2002; Unuma et al., 2003). Nutritive phagocytes are also responsible for phagocytizing relict germ cells after spawning (Holland and Giese, 1965). Thus, during gametogenesis, the gonad may exhibit a variety of changes in major cell populations (Fuji, 1960; Byrne, 1990; Walker et al., 1998; Unuma, 2002) and biochemistry (Chatlynne, 1969; Montero-Torreiro and Garcia-Martinez, 2003; Unuma et al., 2003).

Since many echinoids exhibit an annual gonad cycle, seasonal changes in biotic and abiotic factors may play an important role in regulating the cycle. Food availability, photoperiod, and temperature can significantly influence the reproductive cycles of sea urchins (Pearse et al., 1986; Yamamoto et al., 1988; Ito et al., 1989; Sakairi et al., 1989; Pearse and Cameron, 1991; Walker and Lesser, 1998; Spirlet et al., 2000; Garrido and Barber, 2001; Kelly, 2001; Shpigel et al., 2004). Pearse and Cameron (1991) concluded that the effect of temperature on the gametogenic cycle in echinoids is species specific.

In this study, *L. variegatus* were exposed to three temperature treatments similar to average field temperatures of winter, spring/fall, and summer seasons for 8 wk. To determine the direct effect of temperature on the progression of gametogenesis and nutrient storage, individuals were collected in the fall when the gonads are small and gamete production is minimal. The effect of temperature on cell populations in the gonad was evaluated by tissue histology and image analysis, and the proximate composition of the gonad was determined.

MATERIALS AND METHODS

Collection and maintenance of sea urchins.—*Lytechinus variegatus* (ca. 35.63 ± 1.24 g wet weight and 40-mm diameter) were collected in October 2001 from Saint Joseph Bay, FL (30°N, 85.5°W) and were transported to the University of Alabama at Birmingham in coolers containing 12 liters of saltwater with aeration. The water temperature at collection was 20°C. Sea urchins were placed into nine 80-liter aquaria (n = eight sea urchins per aquarium) maintained in enclosed incubators (three aquaria per incubator). Individuals were held at 22°C for 1 wk before the 8-wk study. Incubator temperatures were then adjusted to one of three experimental temperatures (16, 22, or 28°C) over a 24-hr period. Experimental temperatures and photoperiod (12 hr:12 hr, light:dark) were maintained throughout the study. Sea urchins were main-

tained individually in 1-liter polyethylene containers placed inside the 80-liter aquaria with recirculating 100% synthetic seawater (Instant Ocean Sea Salt, 32 ppt salinity) delivered through a manifold system within each aquarium (Hofer, 2002). Flow rate within the individual containers was 1 liter/3 min. Salinity was checked daily and adjusted by adding diluted synthetic seawater as needed. Each week, the water in each tank was circulated through an ultraviolet filter for 24 hr. Ammonia, nitrite, and nitrate concentrations (<0.5 mg/L) and pH (ca. 8–8.3) were maintained within normal culture parameters.

Feed and feed preparation.—The nutrient composition of a formulated dry semipurified feed is reported in Table 1. This feed was constituted into a wet pellet by adding 10 g of dry formulated feed to a solution of heated seawater (88 ml, 60–70°C, 40 ppt salinity) containing 2 g of agar binder. The wet slurry was cooled, allowed to solidify, and cut into blocks ca. 1 × 2 × 2 cm. The resulting dry matter protein concentration of the constituted feed was calculated to be 21% (Table 1). Fresh food was prepared every 4 d and stored at 4°C for later use. Each individual sea urchin was fed daily the formulated feed ad libitum for 8 wk.

Dissection.—At the beginning of the study, an initial subsample of 25 sea urchins was dissected, and organs were removed and weighed. At the end of 8 wk, all experimental animals were dissected. Gonads were blotted and weighed (organismal wet weights, test diameters, and gonad weights were reported previously by Hofer [2002]). Sex was determined by using a compound light microscope to examine a gonad squash for the presence of sperm or eggs. A subsample of the gonad was placed in Bouin's fixative for subsequent histological analysis. All remaining gonad samples were dried in an oven at 65°C for several days until constant weight and the dried samples were weighed. For the determination of temperature effect on gonad weight, an ANCOVA was performed on gonad wet weight data using total individual wet weight minus the gonad wet weight as a covariate. Means adjusted for body size were compared with Tukey's adjustment for multiple comparison, and significance was reported when $P < 0.05$.

Histology and image analysis.—A subsample of gonad tissue from each sea urchin in each temperature treatment was preserved in Bouin's fixative and subsequently sectioned for histolog-

TABLE 1.   Proximate analysis and calculated composition (percentage dry weight) of the feed formulation.

	Calculated formulation[a]	Calculated constituted feed[b]	Assayed levels[c]
Moisture	5.29	-	-
Crude protein	32.09	20.67	-
Soluble protein	-	-	10.12
Crude fat	9.82	6.33	8.18
Carbohydrate	37.50	24.16	31.53
Crude fiber	6.66	16.79	-
Total ash	8.66	28.65	26.99

[a] Calculated values of the formulated dry diet before incorporation into the food pellet.
[b] Constituted feed consisted of 10% formulation, 2% agar binder, and 88% salt water (40 ppt). Values represent calculated composition after dehydration to constant dry weight.
[c] Values determined by chemical assay for soluble protein, total lipid, and soluble carbohydrate in the constituted feed after dehydration to constant dry weight.

ical analysis. One to three paraffin step sections were mounted for each subsample and were stained with hematoxylin and eosin. Slides were initially examined with a compound light microscope and sex was verified by the presence of sperm or eggs. Individuals were categorized into reproductive stage using the classification method described by Cunningham (2007) for *L. variegatus*: stage I, spent (residual gametes and a thin border of nutritive phagocytes); stage II, renewal (thin layer of germ cells along germinal epithelium and network of nutritive phagocytes across lumen); stage III, growing (nutritive phagocytes fill lumen and maturing germ cells replace nutritive phagocytes in lumen); stage IV, mature (mature gametes fill lumen and only a thin layer of nutritive phagocytes along acinus wall).

Slides from female and male sea urchins were examined using a compound light microscope, and individual acini were photographed at ×10 magnification with a Polaroid digital microscope camera and Adobe Photoshop software. For females, an image analysis program, Optimus 6.51 (Media Cybernetics, L.P., Bethesda, MD), was used to measure the long diameters of a minimum of 50 oocytes per individual to the nearest 1 µm. Only oocytes along the germinal epithelia wall or those clearly distinguishable as eggs (possessing visible nuclei) were measured. Size-frequency distributions of oocyte diameters were analyzed with a Kruskal–Wallis test to determine differences among temperature treatments.

The volume (area) of the acinus occupied by oocytes and germinal epithelium was estimated using Optimus 6.51, and the acinus volume occupied by nonstaining regions (empty space) was measured using Image Tool 2.0 (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX). Area values were then converted to percentages on the basis of the total volume of the individual acinus. The acinus volume occupied by nutritive

phagocytes was then determined indirectly by subtracting the sum of oocyte, germinal epithelium, and nonstaining region percentages from 100%. The percentage volume of oocytes, germinal epithelium, nutritive phagocytes, and nonstaining regions were compared among temperature treatments. For determination of differences among treatments for percentage germinal epithelium and the nonstaining regions, an ANOVA was performed. Welch's variance-weighted ANOVA was performed on percentage of nutritive phagocytes to determine differences among treatments. A Kruskal–Wallis test was performed on the percentage of oocytes to determine differences among treatments.

For males, the volume of the acinus occupied by gametes and nonstaining regions was estimated using Image Tool 2.0, and the volume values were converted to percentages on the basis of the total volume of the individual acinus. Percentage of the acinus occupied by nutritive phagocytes was determined indirectly by subtracting the sum of gamete and nonstaining region percentages from 100%. The percentage volume of gametes, nutritive phagocytes, and nonstaining regions was compared among temperature treatments. For determination of differences among treatments for percentage nutritive phagocytes, an ANOVA was performed. A Kruskal–Wallis test was performed on percentage gametes and nonstaining regions to determine differences among treatments.

Biochemical analysis.—Dried gonad samples from females and males of each treatment were ground to a fine powder using a Wiley mill. Water content was determined by subtraction. The amount of protein soluble in 1 N NaOH in the dried gonad tissue was determined using a spectrophotometric protein assay (Lowry et al., 1951). Amount of total lipid in the dried gonad tissue was determined using a gravimetric lipid

TABLE 2.   Total wet weights and test diameters of sea urchins held at 16, 22, or 28°C for 8 wk. Values represent means ± SE (n = 19–22).[a]

	Temperature		
	16°C	22°C	28°C
Total wet weight (g)			
Initial	35.81 ± 1.33[A]	35.84 ± 1.32[A]	35.23 ± 1.08[A]
Final	44.16 ± 1.34[A]	50.38 ± 1.65[B]	44.72 ± 1.09[A]
% Weight gain	24.39 ± 2.99[A]	41.40 ± 2.92[B]	28.27 ± 3.61[A]
Test diameter (mm)			
Initial	42.08 ± 0.57[A]	42.01 ± 0.46[A]	41.77 ± 0.39[A]
Final	44.10 ± 0.45[A]	46.15 ± 0.49[B]	45.26 ± 0.32[AB]
% Increase	4.93 ± 0.82[A]	9.93 ± 0.96[B]	8.49 ± 0.99[B]

[a] Mean values with same letter within a row indicate no significant difference among temperature treatments (ANOVA, $P > 0.05$).

extraction analysis (Folch et al., 1957). The amount of carbohydrate soluble in reagent-grade H_2SO_4 in the dried gonad tissue was determined using a spectrophotometric carbohydrate assay (Dubois et al., 1956). Dried gonad samples were ashed in a muffle furnace at 500°C for 4.5 hr to obtain total ash content. The insoluble component, assumed to be primarily protein insoluble in 1 N NaOH, was determined by subtracting total lipid, ash, base-soluble protein, and acid-soluble carbohydrate from the total dry weight of the gonad. For the levels of protein, lipid, carbohydrate, and ash in the gonad, an ANCOVA was performed using the total dry gonad weight as a covariate. Means adjusted for the size of the gonad were compared with Tukey's adjustment for multiple comparison, and significance was reported when $P < 0.05$.

Statistical summary.—Statistical analyses were completed using the SAS System 9.0 for Windows (SAS Institute, Cary, NC). Normality and equality of variances were tested before analysis using Kolmogorov–Smirnov and Levene tests, respectively. If the data were normal, parametric tests including ANOVA and ANCOVA were performed to evaluate the effect of temperature. When the null hypothesis was rejected, Tukey's adjustment for multiple comparisons was used to compare each pair of group means. For non-normal data, a Kruskal–Wallis test was performed to evaluate the effect of temperature. Normal data with unequal variances were evaluated using Welch's variance-weighted ANOVA to evaluate the effect of temperature. For all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Organismal and gonad growth.—Total wet weights and diameters of sea urchins did not vary among temperature treatments at the

beginning of the study (Table 2). Significant weight gain was observed for individuals at all temperatures at the end of the 8-wk study period; however, weight gain was highest for individuals held at 22°C (Table 2). Gonad wet and dry weights did not differ between females and males, and values were combined for analysis. Average initial gonad wet and dry weights were 0.38 ± 0.04 and 0.11 ± 0.01 g, respectively. At the end of 8 wk, gonad weights were highest for those individuals held at the 22°C treatment (Table 3). Water content was highest for individuals held at 16°C (Table 3).

Cellular composition.—Reproductive stages for both females and males were identified as stage II, renewal or stage III, growing. Sixty percent of females held at 22°C had advanced to stage III, whereas only 43% of females held at 16 or 28°C had advanced to stage III. Of males held at 22°C, 62.5% had advanced to stage III. Only 25% of males held at 16°C had advanced to stage III, whereas 50% of males held at 28°C had advanced to stage III.

Neither females nor males exhibited a significant relationship between the volume of gametes within an acinus and the size of the gonads (ANCOVA, $P > 0.05$). The volume of the acinus occupied by the germinal epithelium and oocytes was highest in females held at 22°C (Figs. 1A, 2A). Similarly, the volume of the acinus occupied by nutritive phagocytes was lowest in these individuals. Overall, oocyte diameters were lowest for females held at 28°C (Fig. 3). The largest percentage of oocytes with a diameter greater than 100 μm was observed in females held at 22°C (6.8, 9.6, and 3.2% at 16, 22, and 28°C, respectively). In males, the volume of acinus occupied by gametes was lowest for males held at 28°C, whereas the volume of acinus occupied by nutritive phagocytes was highest for males held at 28°C (Figs. 1B, 2B).

TABLE 3. Gonad weights and water content of an initial subsample and of sea urchins held at 16, 22, or 28°C for 8 wk. Values represent actual means ± SE with means adjusted for weight ± SE within parentheses (n = 19–25).[a]

		Temperature		
	Initial	16°C	22°C	28°C
Wet weight (g)	0.38 ± 0.04	4.56 ± 0.24	5.97 ± 0.34	3.95 ± 0.40
		(4.71 ± 0.30[A])	(6.05 ± 0.29[B])	(3.97 ± 0.30[A])
Dry weight (g)	0.11 ± 0.01	1.34 ± 0.07	1.93 ± 0.10	1.34 ± 0.13
		(1.37 ± 0.09[A])	(1.96 ± 0.09[B])	(1.35 ± 0.09[A])
Water content (%)	71.5 ± 0.86	70.7 ± 0.41[B]	67.4 ± 0.47[A]	65.8 ± 0.66[A]

[a] Means adjusted for weight (total weight – weight of gonad tissue [mg]) with same letter within a row indicate no significant difference among temperature treatments (ANCOVA, $P > 0.05$). Actual mean values with same letter within a row indicate no significant difference among temperature treatments (ANOVA, $P > 0.05$).

Biochemical composition of the gonad.—The proximate content of the gonad differed between sexes; therefore, proximate content data were separated by sex for analysis. In ovaries, the amount of soluble protein was highest at 22°C, but did not differ significantly between 16 or 28°C (Table 4). The amounts of soluble carbohydrate, total lipid, and ash in the ovaries did not differ among the temperature treatments. In testes, the amount of lipid was highest at 16°C, but did not differ between 22 or 28°C (Table 5). The amounts of soluble protein, soluble carbohydrate, and ash in the testes did not differ among the temperature treatments.

DISCUSSION

Exposure temperature significantly affected cellular populations within the gonads of adult *L. variegatus* under the conditions of this study. Gamete production was favored in individuals held at median temperatures. In females held at median temperatures, large oocytes and proliferative activity along the germinal epithelium suggest that nutrients were allocated to both nutritive phagocytes and gamete production. Females exposed to low or high temperatures used a different nutrient allocation strategy. At low and high temperatures, females continued to store nutrients in nutritive phagocytes, but minimized gamete production. In males, the numbers of gametes found along the germinal epithelium varied greatly within all temperature treatments; however, males in both low and median temperatures began to allocate nutrients for the production of sperm while gamete production was significantly reduced at high temperatures.

Temperature has been correlated with a variety of effects on the reproduction of echinoids, and these effects are typically species specific. Information on the direct or indirect effects of temperature on gamete development in *L. variegatus* is limited. Spawning events for *L. variegatus* typically occur in mid-spring and to a lesser extent in late summer (reviewed by Watts et al., 2001), which would suggest that gamete production is greatest during fall and winter months of low seawater temperature and is reduced in summer months when high seawater temperatures prevail. Moore and Lopez (1972) observed that high seawater temperatures negatively affected the spawn output of *L. variegatus* in a population along the coast of Miami, FL. High seawater temperatures have been shown to influence gonad maturation in several sea urchin species. Gonad maturation in the Japanese sea urchin *Hemicentrotus pulcherrimus* was promoted by lowering the water temperature from 26°C to 15°C, and high water temperatures inhibited gametogenesis in the sea urchin *Pseudocentrotus depressus* (Ito et al., 1989; Sakairi et al., 1989; Yamamoto et al., 1988). Garrido and Barber (2001) found that low water temperature promoted oocyte development in *Strongylocentrotus droebachiensis*. Low water temperature, however, inhibited maturation of the gonad in *Anthocidaris crassispina* (Sakairi et al., 1989).

Nutritive phagocytes comprised a majority of the volume of the acini in the gonad tissue for all individuals in all temperature treatments. This finding suggests that nutrient deposition occurs rapidly and preferentially into nutritive phagocytes when the initial gonad size is relatively small and nutrients are not limiting; however, the ultrastructure of the nutritive phagocytes in both females and males differed substantially between the low- and high-temperature treatments. At the lower temperature, nutritive phagocytes extended throughout the lumen, were highly vacuolated, and were correlated with high moisture content of the gonad. At the highest temperature, nutritive phagocytes were eosin-rich and contained very few vacuoles. Nutritive phagocytes from individuals held at the median temperature showed intermediate morphologies. These mor-
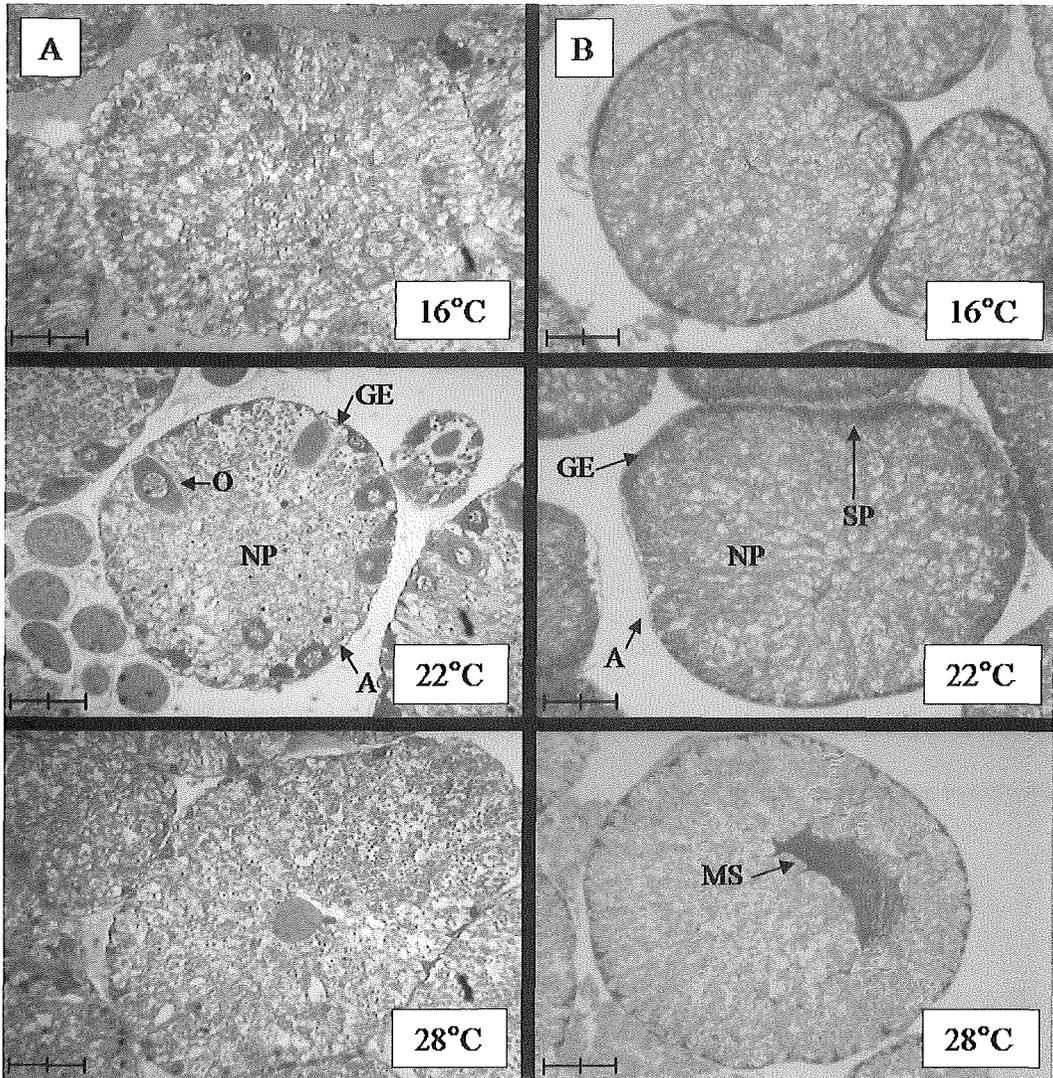
Fig. 1.   Gonad histology. (A) Histological cross-sections of female acini from 16, 22, or 28°C stained with hematoxylin and eosin. (B) Histological cross-sections of male acini from 16, 22, or 28°C stained with hematoxylin and eosin. Scale bars represent 200 μm. A = acinus, NP = nutritive phagocyte, GE = germinal epithelium, O = oocyte, SP = spermatocyte, MS = mature spermatozoa.

phological differences suggest temperature-dependent differences in the pattern of nutrient storage within the nutritive phagocytes.

Seasonal changes in the morphology of nutritive phagocytes during reproductive cycles have been observed in some sea urchin species. Holland and Giese (1965) reported that the nutritive phagocytes in *Strongylocentrotus purpuratus* alternated between two different morphologies during the reproductive cycle. From July to December, the nutritive phagocytes contained numerous cytoplasmic globules that stained strongly with eosin: globulated phase. During the globulated phase, gametes in both female

and male *S. purpuratus* were maturing. From January to July, the nutritive phagocytes were in a deglobulated phase where the cytoplasmic globules were scarce and nutritive phagocytes appeared vacuolated. The deglobulated phase was often noted when gonads were approaching spawning or were postspawn. Chatlynne (1969) also noted in *S. purpuratus* a period of increasing globulation in nutritive phagocytes from August to January when oocytes were developing and maturing and decreasing globulation or emptying occurring from January to August when oocytes were most mature and spawning. Nicotra and Serafino (1988) described similar changes in
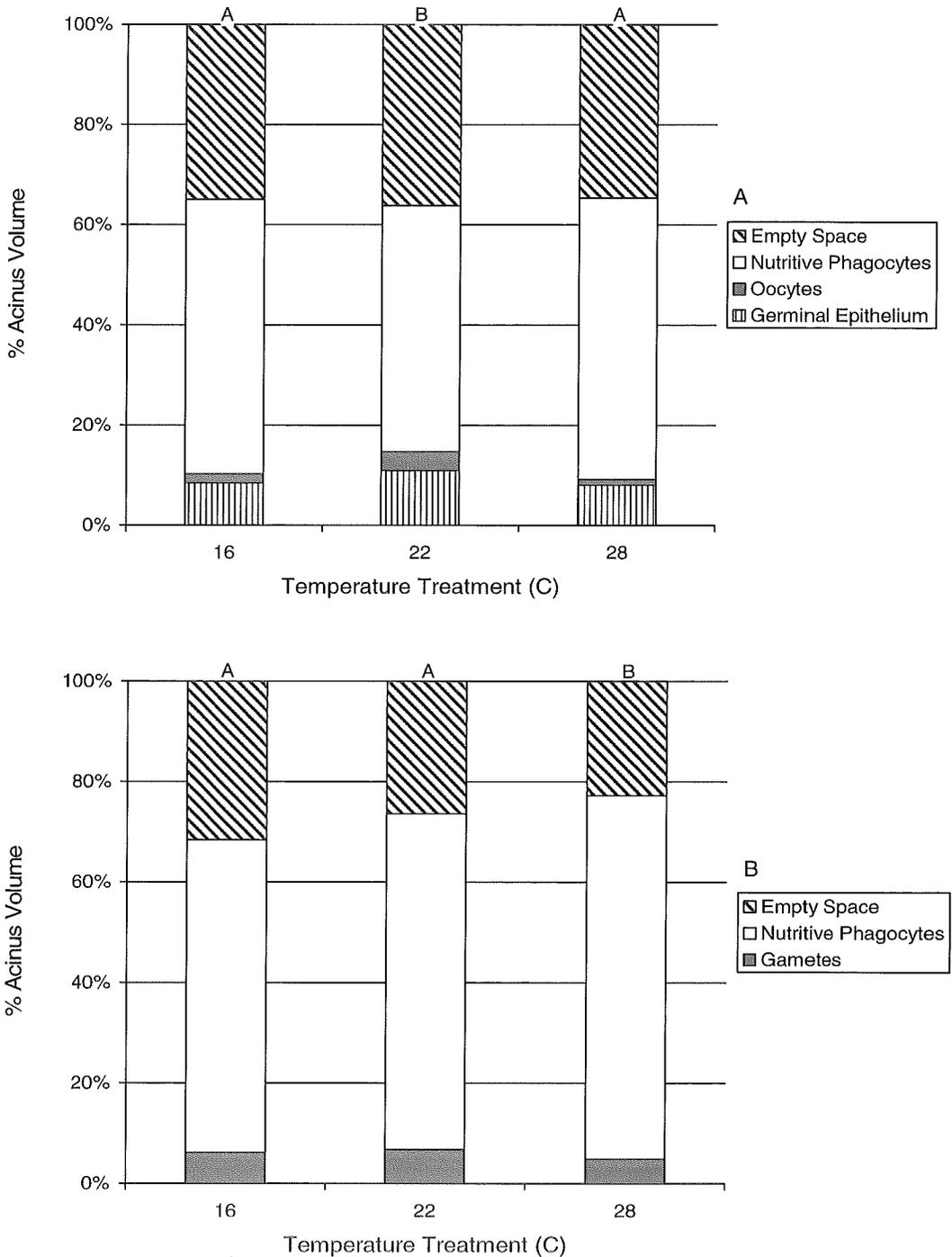
Fig. 2.   Cellular composition of the gonad. (A) Percentage volume of nonstaining regions (empty space), nutritive phagocytes, oocytes, and germinal epithelium within acini of ovaries of females held at 16, 22, or 28°C for 8 wk (n = 5–7 sea urchins; mean of 44 acini per temperature). (B) Percentage volume of nonstaining regions (empty space), nutritive phagocytes, and gametes within acini of testes of males held at 16, 22, or 28°C for 8 wk (n = 12–15 sea urchins, mean of 73 acini at each temperature). Same letters indicate no significant difference among treatments for nutritive phagocytes (ANOVA, *P* > 0.05).
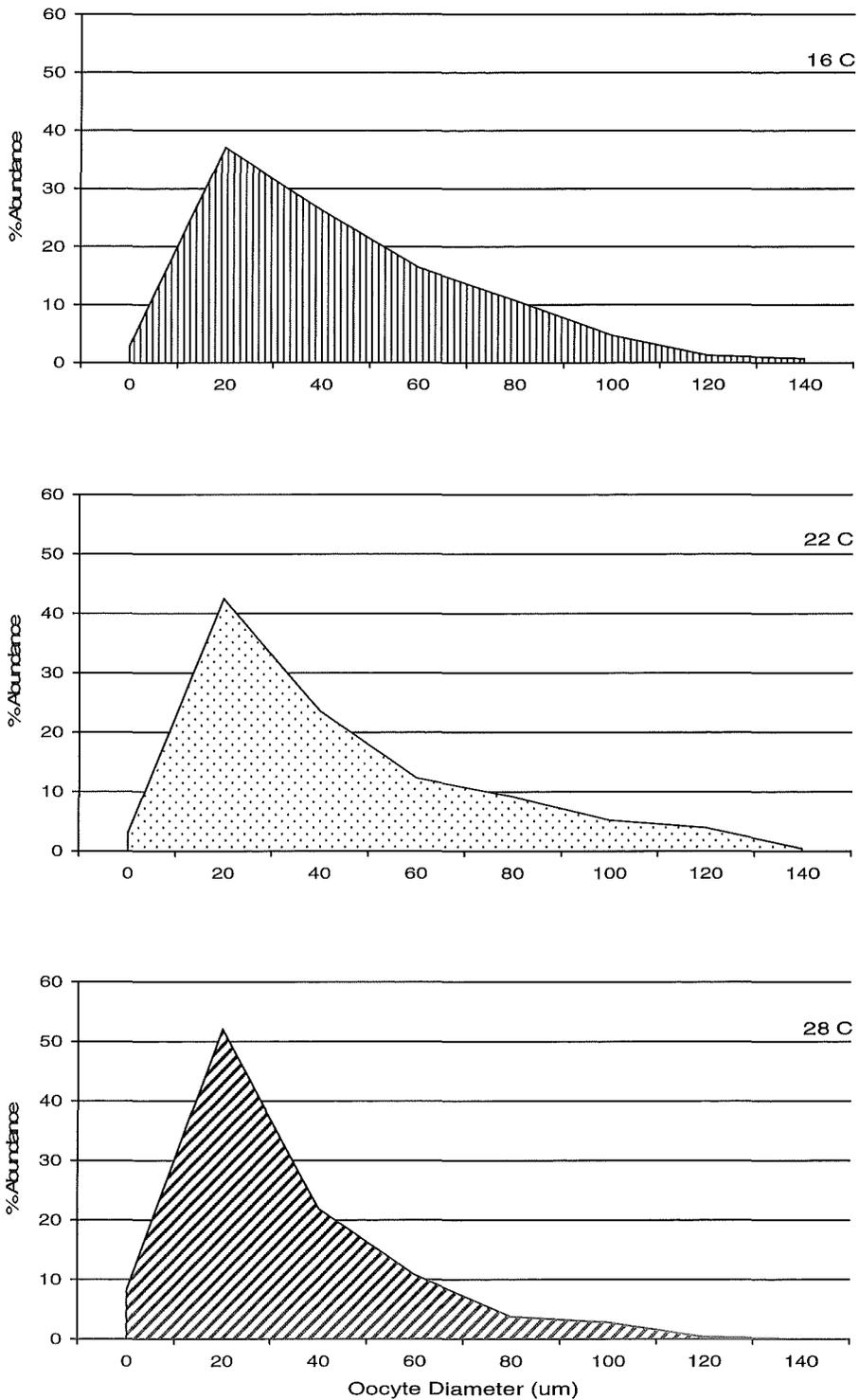
Fig. 3.   Oocyte diameters. Size-frequency distribution of oocytes in female sea urchins held at 16, 22, or 28°C for 8 wk (n = 5–7 sea urchins, 510 oocytes at each temperature).

TABLE 4. Proximate composition of the gonad tissue for females held at 16, 22, or 28°C for 8 wk. Values represent actual means ± SE with means adjusted for weight ± SE within parentheses (n = 5–7).[a]

	Temperature		
	16°C	22°C	28°C
Females			
Soluble protein (mg)	310.96 ± 24.10	450.51 ± 57.32	322.55 ± 46.28
	(342.96 ± 7.10[A])	(375.92 ± 8.41[B])	(343.83 ± 7.10[A])
Insoluble protein (mg)	139.48 ± 15.94	214.33 ± 33.34	142.16 ± 23.48
	(155.10 ± 11.70[A])	(177.90 ± 13.85[A])	(152.55 ± 11.70[A])
Total lipid (mg)	299.10 ± 16.00	402.76 ± 43.37	304.56 ± 44.12
	(325.68 ± 9.55[A])	(340.80 ± 11.30[A])	(322.23 ± 9.55[A])
Carbohydrate (mg)	488.72 ± 57.54	595.06 ± 67.35	519.30 ± 82.60
	(541.04 ± 21.63[A])	(473.09 ± 25.60[A])	(554.10 ± 21.63[A])
Ash (mg)	67.60 ± 4.42	70.51 ± 10.74	61.81 ± 7.53
	(71.01 ± 2.19[A])	(61.63 ± 2.90[A])	(63.48 ± 2.19[A])

[a] Means adjusted for weight (total dry gonad weight [mg]) with same letter within a row indicate no significant difference among temperature treatments (ANCOVA, $P > 0.05$).

the nutritive phagocytes of the sea urchin *Paracentrotus lividus* where globulated nutritive phagocytes were common during summer months. Globulation decreased during fall and winter, and deglobulated nutritive phagocytes were common in spring. Overall, globulation of nutritive phagocytes appeared to be most common during months of high temperatures, whereas deglobulation occurs during months of low temperatures. This trend has also been observed in *L. variegatus* in St. Joseph Bay, FL (Cunningham, pers. obs.).

Relatively low volumes of gametes were observed for all sea urchins regardless of temperature treatment after 8 wk of feeding. Mature gonads are not commonly observed in *L. variegatus* obtained from field populations during late fall; consequently, gamete production

may be limited when sea urchins are collected and held out of season. Alternatively, 8 wk may not allow sufficient time for the complete development and maturation of the gametes, even when individuals are held in favorable water temperatures and fed adequate diets.

Growth of the gonad tissue was primarily due to the accumulation of nutrients in the nutritive phagocytes. Weight gain of gonad tissue was highest for individuals held at the median temperature. Differences in gonad weight are often attributed to food availability or quality (Garrido and Barber, 2001; Spirlet et al., 2001); however, food was not limited in this study and was homogeneous in quality. We hypothesize that reduced production of gonad tissue at the high temperature was the result of the increased metabolic costs associated with increasing tem-

TABLE 5. Proximate composition of the gonad tissue for males held at 16, 22, or 28°C for 8 wk. Values represent actual means ± SE with means adjusted for weight ± SE within parentheses (n = 12–15).[a]

	Temperature		
	16°C	22°C	28°C
Males			
Soluble protein (mg)	340.25 ± 25.83	558.43 ± 40.29	353.11 ± 41.66
	(404.60 ± 10.45[A])	(442.08 ± 9.34[A])	(417.97 ± 9.34[A])
Insoluble protein (mg)	111.48 ± 14.31	243.40 ± 31.85	193.83 ± 21.41
	(126.47 ± 14.28[A])	(146.80 ± 12.77[A])	(220.42 ± 12.77[B])
Total lipid (mg)	337.60 ± 28.85	429.94 ± 26.72	262.37 ± 33.87
	(387.70 ± 10.46[B])	(339.36 ± 9.36[A])	(312.88 ± 9.36[A])
Carbohydrate (mg)	481.54 ± 38.24	657.30 ± 30.98	460.37 ± 55.86
	(565.34 ± 17.56[A])	(572.37 ± 15.70[A])	(541.95 ± 15.70[A])
Ash (mg)	82.78 ± 5.27	108.66 ± 6.71	82.13 ± 8.33
	(93.95 ± 3.41[A])	(88.48 ± 3.05[A])	(93.39 ± 3.05[A])

[a] Means adjusted for weight (total dry gonad weight [mg]) with same letter within a row indicate no significant difference among temperature treatments (ANCOVA, $P > 0.05$).

peratures, and reduced production of gonad tissue at low temperature was most likely the result of decreased food consumption rates during low-temperature acclimation (Hofer, 2002). An effect of temperature on gonad production has been noted in *P. lividus* where, for similar food intake, higher temperatures yielded higher gonad growth (Spirlet et al., 2000). In contrast, McBride et al. (1997) found that temperature had little effect on gonad production in *Strongylocentrotus franciscanus*, although the difference between the temperatures tested was limited (12.0°C vs 16.1°C).

Temperature-dependent nutrient absorption has been shown in *L. variegatus* (Gibbs and Watts, 2004), and the amount of proximate nutrients assimilated in the gonad tissue was affected by temperature in this study. High protein levels in females held at the median temperature can be attributed to higher amounts of gametic tissue within the ovaries. The morphology of the nutritive phagocytes suggests that the mechanism of nutrient storage differed among temperature treatments. In males, the morphological differences of nutritive phagocytes were directly correlated with the amount of lipid in the gonad. At the low temperature, nutritive phagocytes were highly vacuolated and the amount of lipid was highest, and at high temperatures, nutritive phagocytes were globulated and the amount of lipid was lower.

The biochemistry of the gonad in many sea urchin species is commonly correlated with changes in the reproductive cycle. In *Pseudocentrotus depressus* and *S. purpuratus*, the concentrations of lipids and carbohydrates decreased in the gonad as gametogenesis progressed and gametes matured (Unuma et al., 2003; Chatlynne, 1969). Levels of proteins and nucleic acids were observed to increase in *P. depressus* gonads as gametogenesis progressed and gonad index increased (Unuma et al., 2003). Similarly, Montero-Torreiro and Garcia-Martinez (2003) observed that in *Paracentrotus lividus* gonads, concentrations of glycogen decreased with the progression of gametogenesis, and protein concentrations increased with increasing gonad index. In *L. variegatus,* carbohydrate levels in the gonad peaked in fall and decreased throughout the winter and spring, whereas protein levels were lowest in fall and reached a maximum in winter and early spring (Cunningham and Watts, 2005). Variation in levels of lipids in *L. variegatus* gonads did not correlate with season; however, lipid levels were higher in females than in males (Cunningham and Watts, 2005). The reproductive state of the sea urchin may exert a greater influence on proximate composition of the gonad than acclimation temperature.

In this study, individuals were allowed to acclimate to their respective test temperature during the course of the 8-wk experiment. Hofer (2002) suggested that 4 to 5 wk were necessary for acclimation to occur. Consequently, some of the differences observed in this study could be attributed to physiological changes during the acclimation period. An alternative approach would be to acclimate individuals at their respective test temperature before the initiation of the study. It would be very difficult, however, to standardize the initial nutritional condition of a population of sea urchins, and further comparisons would be confounded.

Under the conditions of this study, 8 wk was sufficient to induce significant increases in gonad weight. Most of the weight gain was attributed to increased volumes of nutritive phagocytes. Temperature-dependent gamete production was observed; however, additional effects may be observed if sea urchins are held for longer periods of time, at different reproductive seasons, or at different photoperiods. The low and high exposure temperatures evaluated in this study are well within the normal limits experienced by *L. variegatus* (Beddingfield and McClintock, 2000). We suggest that the absolute effect of temperature on gonad production will be species specific and will depend on the energetic balance among food acquisition, metabolic maintenance costs, and production (assimilation) costs. We hypothesize that exposure of *L. variegatus* to more extreme winter or summer temperatures may induce additional changes in cell populations and biochemical composition.

Lytechinus variegatus is an important species in many of the nearshore communities within the Gulf of Mexico, particularly in ecosensitive habitats populated by seagrasses. Long-term changes in temperature can result in changes in nutrient storage and, as a consequence, reproductive success of the species. Temperature-mediated effects on *L. variegatus* reproduction that translate to the population level may, in turn, influence community structure and the success of other species inhabiting seagrass communities.

LITERATURE CITED

BEDDINGFIELD, S. D., AND J. B. McCLINTOCK. 2000. Demographic characteristics of *Lytechinus variegatus* (Echinoidea: Echinodermata) from three habitats in a north Florida Bay, Gulf of Mexico. Mar. Ecol. 21:17–40.

BYRNE, M. 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Mar. Biol. 104:275–289.

CHATLYNNE, L. G. 1969. A histochemical study of oogenesis in the sea urchin, *Strongylocentrotus purpuratus*. Biol. Bull. 136:167–184.

CUNNINGHAM, A. W. 2007. The reproductive cycle of *Lytechinus variegatus* (Echinodermata: Echinoidea) on the Florida Panhandle. PhD diss., University of Alabama at Birmingham.

———, AND S. A. WATTS. 2005. Proximate composition of the gonad varies during the reproductive cycle of *Lytechinus variegatus*. J. Ala. Acad. Sci. 76:105.

DuBois, M., K. A. GILLES, J. K. GILLES, J. K. HAMILTON, P. A. REBERS, AND R. SMITH. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350–356.

ERNEST, R. G., AND N. J. BLAKE. 1981. Reproductive patterns within sub-populations of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinodea). J. Exp. Mar. Biol. Ecol. 55:25–37.

FOLCH, J., M. LEES, AND G. H. SLOANE STANLEY. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497–509.

FUJI, A. 1960. Studies on the biology of the sea urchin I. Superficial and histological gonadal changes in the gametogenic process of two sea urchins, Strongylocentrotus nudus and Strongylocentrotus intermedius. Bull. Fac. Fish. Hokkaido Univ. 11:1–14.

GARRIDO, C. L., AND B. J. BARBER. 2001. Effects of temperature and food ration on gonad growth and oogenesis of the green sea urchin, *Strongylocentrotus droebachiensis*. Mar. Biol. 138:447–456.

GIBBS, V. K., AND S. A. WATTS. 2004. Exposure temperature affects nutrient absorption in *Lytechinus variegatus*, p. 187–192. *In:* Echinoderms: Müchen. T. Heinzeller and J. H. Nebelsick (eds.). A.A. Balkema Publishers, Leiden, the Netherlands.

HOFER, S. C. 2002. The effect of temperature on feeding and growth characteristics of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). Master's thesis, University of Alabama at Birmingham.

HOLLAND, N. D., AND A. C. GIESE. 1965. An autoradiographic investigation of the gonads of the purple sea urchin (*Strongylocentrotus purpuratus*). Biol. Bull. 128:241–258.

ITO, S., M. SHIBAYAMA, A. KOBAYAKAWA, AND Y. TANI. 1989. Promotion of maturation and spawning of sea urchin *Hemicentrotus pulcherrimus* by regulating water temperature. Nippon Suisan Gakkaishi 55:757–763.

KELLY, M. S. 2001. Environmental parameters controlling gametogenesis in the echinoid *Psammechiunus miliaris*. J. Exp. Mar. Biol. Ecol. 266:67–80.

KLINGER, T. S., H. S. HSIEH, R. A. PANGALLO, C. P. CHEN, AND J. M. LAWRENCE. 1986. The effect of temperature on feeding, digestion and absorption of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). Physiol. Zool. 59:332–336.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with Folin reagent. J. Biol. Chem. 193:265–275.

McBRIDE, S. C., W. D. PINNIX, J. M. LAWRENCE, A. L. LAWRENCE, AND T. M. MULLIGAN. 1997. The effect of temperature on production of gonads by the sea urchin *Strongylocentrotus franciscanus* fed natural and prepared diets. J. World Aquat. Soc. 28:357–365.

MONTERO-TORREIRO, M. F., AND P. GARCIA-MARTINEZ. 2003. Seasonal changes in the biochemical composition of body components of the sea urchin, *Paracentrotus lividus*, in Lorbé (Galicia, north-western Spain). J. Mar. Biol. Assoc. U. K. 83:575–581.

MOORE, H. B., T. JUTARE, J. C. BAUER, AND J. A. JONES. 1963. The biology of *Lytechinus variegatus*. Bull. Mar. Sci. 13:23–53.

———, AND N. N. LOPEZ. 1972. Factors controlling variation in the seasonal spawning pattern of *Lytechinus variegatus*. Mar. Biol. 14:275–280.

———, AND B. F. McPHERSON. 1965. A contribution to the study of the productivity of the urchins *Tripneustes esculentus* and *Lytechinus variegatus*. Bull. Mar. Sci. 15:855–871.

NICOTRA, A., AND A. SERAFINO. 1988. Ultrastructural observations on the interstitial cells of the testis of *Paracentrotus lividus*. Int. J. Invert. Reprod. Dev. 13:239–250.

PEARSE, J. S., AND R. A. CAMERON. 1991. Echinodermata: Echinoidea, p. 513–662. *In:* Reproduction of marine invertebrates: echinoderms and lophorates A. C. Giese, J. S. Pearse, and V. B. Pearse (eds.). Vol. VI. Boxwood Press, Pacific Grove, CA.

———, V. B. PEARSE, AND K. K. DAVIS. 1986. Photoperiodic regulation of gametogenesis and growth in the sea urchin *Strongylocentrotus purpuratus*. J. Exp. Zool. 237:107–118.

SAKAIRI, K., M. YAMAMOTO, K. OHTSU, AND M. YOSHISA. 1989. Environmental control of gonadal maturation in laboratory-reared sea urchins, *Anthocidaris crassispina* and *Hemicentrotus pulcherrimus*. Zoolog. Sci. 6:721–730.

SHPIGEL, M., S. C. McBRIDE, S. MARCIANO, AND I. LUPATSCH. 2004. The effect of photoperiod and temperature on the reproduction of European sea urchin *Paracentrotus lividus*. Aquaculture 232:343–355.

SPIRLET, C., P. GROSJEAN, AND M. JANGOUX. 2000. Optimization of gonad growth by manipulation of temperature and photoperiod in cultivated sea urchins, *Paracentrotus lividus* (Lamarck) (Echinodermata). Aquaculture 185:85–99.

———, ———, AND M. JANGOUX. 2001. Cultivation of *Paracentrotus lividus* (Echinodermata: Echinoidea) on extruded feeds: digestive efficiency, somatic and gonadal growth. Aquat. Nut. 7:91–99.

UNUMA, T. 2002. Gonadal growth and its relationship to aquaculture in sea urchins, p. 115–127. *In:* The sea urchin: from basic biology to aquaculture. Y. Yokota, V. Matranga, and Z. Smolenicka (eds.). A. A. Balkema Publishers, Lisse, The Netherlands.

————, T. YAMAMOTO, T. AKIYAMA, M. SHIRAISHI, AND H. OHTA. 2003. Quantitative changes in yolk protein and other components in the ovary and testis of the sea urchin *Pseudocentrotus depressus.* J. Exp. Biol. 206:365–372.

WALKER, C. W., AND M. P. LESSER. 1998. Manipulation of food and photoperiod promotes out-of-season gametogenesis in the green sea urchin, *Strongylocentrotus droebachiensis:* implications for aquaculture. Mar. Biol. 132:663–676.

————, N. A. McGINN, L. M. HARRINGTON, AND M. P. LESSER. 1998. New perspectives on sea urchin gametogenesis and their relevance to aquaculture. J. Shellfish Res. 17:1507–1514.

————, T. UNUMA, N. A. McGINN, L. H. HARRINGTON, AND M. P. LESSER. 2001. Reproduction of sea urchins, p. 5–26. *In:* The edible urchin: biology and ecology. J. M. Lawrence (ed.). Elsevier, Amsterdam, the Netherlands.

WATTS, S. A., J. B. McCLINTOCK, AND J. M. LAWRENCE. 2001. The ecology of *Lytechinus variegatus,* p. 375–393. *In:* The edible urchin: biology and ecology. J. M. Lawrence (ed.). Elsevier, Amsterdam, the Netherlands.

YAMAMOTO, M., M. ISHINE, AND M. YOSHIDA. 1988. Gonadal maturation independent of photic conditions in laboratory-reared sea urchins, *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus.* Zoolog. Sci. 5:979–988.

(VKG, SAW) DEPARTMENT OF BIOLOGY, THE UNIVERSITY OF ALABAMA AT BIRMINGHAM, CAMPBELL HALL, BIRMINGHAM, ALABAMA 35294-1170; AND (ALL) TEXAS A&M AGRICULTURAL EXPERIMENT STATION, SHRIMP MARICULTURE RESEARCH LAB, 1300 PORT STREET, PORT ARANSAS, TEXAS 78373. Date accepted: March 18, 2008.