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RESEARCH ARTICLE

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Can photoperiod manipulation affect gonad development of a boreo-arctic echinoid (*Strongylocentrotus droebachiensis*) following exposure in the wild after the autumnal equinox?

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Abstract A laboratory experiment was conducted in the winter of 2003–2004 to assess the effect of varying photoperiod regime on consumption rate, assimilation rate, absorption efficiency, and gonad development of the green sea urchin, *Strongylocentrotus droebachiensis*. Adult individuals were collected from the wild after they had been exposed to the ambient autumn photoperiod cue (which is the extraneous trigger thought to elicit gametogenesis in this species) and placed at ambient temperature for 12 weeks under five different photoperiod regimes: (1) 24 h light:0 h dark = "0D", (2) 16 h light:8 h dark = "8D", (3) 8 h light:16 h dark = "16D", (4) 0 h light:24 h dark = "24D", and (5) ambient photoperiod (range: 10.50–15.25 h dark). Urchins in these five treatments were fed ad libitum with bull kelp, *Nereocystis luetkeana*. A sixth treatment consisted of starved individuals held under 0D conditions. Various gonad factors including gonad index, percent gonad water, gonad colour (CIE lightness or L^*, CIE hue or a^*, and CIE chroma or b^*), percent area occupation of the gonad by various cell types (nutritive phagocytes, spermatozoa, and secondary oocytes/ova), and stage of development were assessed at the beginning of the experiment and at weeks 4, 8, and 12 of the study. Consumption and assimilation rates were assessed at weeks 4 and 12 and absorption efficiency at week 12 of the experiment. Urchins were predominantly in the growing and premature stages at the beginning of the experiment, but by week 4 at least 20% of individuals in all treatments receiving food were classified as mature. Spawning occurred during all these treatments between weeks 4 and 8, as evidenced by significant decreases in spermatozoa and secondary oocytes/ova and a significant decrease in percent gonad water, but was not accompanied by major declines in gonad indices. Greater than 90% of individuals in all five of the fed treatments were in the recovering and growing stages at the end of the experiment. The 16D treatment had by far the greatest percentage of urchins in the growing stage. In contrast, individuals that were starved were predominantly in the mature stage at weeks 4, 8, and 12, with only ~30% reaching the spawning stage by the end of the experiment. Photoperiod significantly affected gonad indices at the termination of the experiment with gonad index being the highest in the 16D treatment; this was significantly greater than in the 8D and ambient treatments. Photoperiod did not significantly affect gonad percent water, gonad lightness, or gonad hue. Gonad chroma was significantly affected by photoperiod, urchins held under ambient conditions having significantly lower b^* readings than individuals in any other treatment. Photoperiod had little or no affect on consumption rate, assimilation rate, or absorption efficiency. Thus, differences among treatments in regards to gonad index, gonad chroma, and stage of development cannot be attributed to variations in feeding, absorption, or assimilation. The results of this experiment indicate that once gametogenesis is initiated, photoperiod manipula-

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tion cannot prevent ultimate spawning. However, photoperiod regime can affect the rate at which urchins move through the various stages of the gametogenic cycle. Urchins placed on short days under artificial lighting (16D) moved through the spawning stage into recovering and growing stages the fastest. This photoperiod regime also produced the highest gonad index at the end of the experiment. Since the commercial urchin market prefers large gonads in the growing and premature stages (i.e. before the mature stage is reached and gonads start leaking sperm and eggs), short day-lengths under artificial lighting (16D) appear to be the best photoperiod conditions for optimizing marketability.

Introduction

The green sea urchin, *Strongylocentrotus droebachiensis*, has a widespread boreo-arctic distribution (Mortensen 1943; Jensen 1974; Bazhin 1998). The species has a major annual spawn that typically occurs at the first phytoplankton bloom in late winter or early to mid-spring (February–June) (Cocanour and Allen 1967; Stephens 1972; Himmelman 1975, 1978; Falk-Petersen and Lønning 1983; Keats et al. 1984; Munk 1992; Meidel and Scheibling 1998; Oganesyan 1998), although minor spawning events may also occur in the summer and fall (Keats et al. 1987; Meidel and Scheibling 1998). As with all echinoids, *S. droebachiensis* is dioecious, but rare cases of hermaphrodites have been observed (Gadd 1907; C.M. Pearce, personal observation). The gonads contain two major types of cells: (1) somatic cells—nutritive phagocytes that are present in both sexes and are used mainly for energy storage (predominantly glycogen) and (2) germinal cells—oogonia that develop into fully mature ova in the ovary and spermatogonia that develop into fully differentiated spermatozoa in the testis (Holland and Giese 1965; Holland 1967; Holland and Holland 1969).

The gonads of both male and female sea urchins pass through a number of developmental changes during the annual reproductive cycle and these changes can be linked to the activities of the somatic and germinal cells within the gonads. Various studies of echinoid reproduction have classified these developmental transitions into a number of predominant stages (Fuji 1960a, b; Chatlynne 1969; Pearse 1969b; Walker and Lesser 1998; Byrne 1990). There are typically five or six developmental stages, depending on the particular classification scheme. There is a recovery stage following spawning when nutritive phagocytes are at their smallest size during the year. Primary oocytes occur along the ascinal walls of the ovary while spermatogonia and primary spermatozoa line the ascinal walls of the testis. Relict ova and spermatozoa from the previous spawning event may still be present in various degrees of lysis. Nutritive phagocytes form a meshwork across the ascinus in both the ovary and testis. The recovery stage is followed by a growing stage where primary oocytes and spermatocytes increase in size and number, although both still occur along the ascinal walls. Nutritive phagocytes fill the ascinus of both the ovary and testis. In the premature stage, the ovary contains oocytes at all stages of development. As vitellogenesis proceeds, large primary oocytes move towards the centre of the ascinus and displace the nutritive phagocytes. Fully developed oocytes undergo maturation and become ova. The premature testis contains spermatocytes along the ascinal wall and spermatozoa that accumulate in the lumen, displacing the nutritive phagocytes. The mature-stage ovary is densely packed with mature ova with a few small oocytes along the ascinal wall. Nutritive phagocytes are absent or form a thin layer around the oocytes. The mature testis is filled with spermatozoa and the nutritive phagocytes are concentrated along the edges of the ascinus. After spawning, individuals are classified into a spent stage. A spent ovary has a thin ascinal wall and appears relatively empty except for the occurrence of relict, unspawned ova and large oocytes. Nutritive phagocytes may occur at the ascinus periphery in a thin layer as do clusters of primary oocytes. A spent testis also has a thin ascinal wall and is relatively devoid of cells except for a thin layer of nutritive phagocytes at the ascinus periphery and some relict spermatozoa.

The cellular mechanisms that stimulate male and female gametogenesis of shallow-water echinoids have been associated with changes in both photoperiod (Boolootian 1963; Holland 1967; Gonor 1973; Pearse et al. 1986a; Bay-Schmith and Pearse 1987; Byrne 1990; McClintock and Watts 1990; King et al. 1994; Hagen 1997; Walker and Lesser 1998; Kelly 2001; Shpigel et al. 2004) and temperature (Chatlynne 1969; Khotimchenko 1982; Yamamoto et al. 1988; Sakairi et al. 1989). Walker and Lesser (1998) showed that the initiation of gametogenesis in *S. droebachiensis* was correlated with decreasing autumn day lengths but that low winter temperatures may be essential for the completion of vitellogenesis (see also Garrido and Barber 2001). *Psammechinus miliaris*, which needs lengthening days for the culmination of gametogenesis, also requires low temperatures for the completion of vitellogenesis (Kelly 2001). Deep-sea echinoids showing reproductive periodicity are believed to use food (pulsed organic flux from surface production) and/or temperature cues for controlling reproductive patterns (Tyler et al. 1982; Tyler and Gage 1984; Gage et al. 1986).

Sea urchins are marketed for their gonads, the bulk of the world's production being shipped to Japan (Sonu 1995; Keesing and Hall 1998). The price that fresh sea-urchin gonads receive on the Japanese fish markets is dependent on a variety of factors including time of year, species of urchin, gonad size, and gonad quality. Quality factors of importance in determining pricing include colour, texture, firmness, and flavour. The urchin market typically prefers bright orange or bright yellow gonads that are firm and sweet tasting. Mature or "ripe" gonads are undesirable since they are generally soft and may leak mature gametes, which is visually unappealing

(Unuma 2002). Thus, the urchin market prefers individuals in the growing or premature stages of development since they are filled with nutritive phagocytes and have few or no mature ova or spermatozoa.

The potential ability to control reproductive development in cultured sea urchins through the manipulation of environmental parameters has major implications for the marketability of cultured product and the profitability of echinoculture. For land-based culture systems, it is hypothesized that the regulation of photoperiod and/or seawater temperatures could inhibit the maturation of sea urchins and enable the marketing of high quality gonads year round. This scenario raises the following question for culturing *S. droebachiensis*. Can the manipulation of environmental parameters inhibit the maturation of gonads or affect maturation, spawning, or post-spawning recovery rates once individuals have been exposed to the fall photoperiod cue? The answer will be of great importance, especially for land-based gonad enhancement operations in which adult urchins are harvested from the wild, kept in captivity, and fed natural and/or prepared feeds in order to increase the quantity and/or quality of their gonads. The present study was initiated to address this question by subjecting adult *S. droebachiensis*—that were collected from the wild after the autumnal equinox (i.e. the date when the sun crosses the celestial equator and night and day are the same length)—to various artificial and natural photoperiod regimes and documenting changes in gonad development.

Materials and methods

Sea-urchin collection and maintenance

Adult green sea urchins (*S. droebachiensis*) were hand collected by SCUBA divers on December 8, 2003 at Gabriola Pass, BC, Canada (49°7′42″N, 123°42′5″W) at a depth of 8–10 m in an urchin barren ground. They were placed in two circular fibreglass holding tanks (diameter × depth: 91.5×76.0 cm), held outdoors under ambient lighting and seawater temperature, and fed periodically with bull kelp (*Nereocystis luetkeana*) until January 17, 2004, the experiment start date. To ensure that urchins for the planned experiment were capable of being reproductively mature, individuals of 40–50 mm test diameter were chosen. Test diameter, test height, and wet weight of 275 urchins were measured and individuals were placed separately in plastic PVC pots (diameter × depth: 10.2×15.0 cm), with one urchin per pot, and supplied with ambient seawater that flowed (mean ± SD: 820 ± 100 ml min^{-1}, $n = 10$) into the bottom of the pot and exited at the top. The seawater temperature, recorded daily, varied from 7.2 to 9.1°C (mean ± SD: 8.20 ± 0.32°C, $n = 64$) during the 12-week experimental period. Fifteen PVC pots were placed in each of 12 fibreglass oval-shaped tanks (length × width × depth: 100×48×45 cm).

Lighting for the experiment was provided by overhead fluorescent lights (Sylvania Super Saver Cool White, 34 W) set to a 24 h light photoperiod. Light intensity at the surface of the PVC pots was measured using a quantum scalar irradiance meter (Biospherical Instruments Inc., CA, USA) and ranged from 1.9×10^{14} to 1.1×10^{15} quanta cm^{-2} s^{-1}. Four different photoperiod regimes were established in the oval-shaped tanks—with daily dark periods of 0 h (0D), 8 h (8D), 16 h (16D), and 24 h (24D)—by using blackout covers placed on top of the tanks. There were three, randomly assigned, oval-shaped tanks for each photoperiod treatment, each tank having 15 urchins housed separately in individual PVC pots. Thus, at the beginning of the experiment, there were 45 urchins per photoperiod treatment. Forty-five urchins, also housed separately in individual PVC pots, were placed outdoors and subjected to ambient lighting conditions.

Sea-urchin feeding and determination of consumption/assimilation rates and absorption efficiency

Urchins were fed ad libitum with bull kelp (*N. luetkeana*) that had been previously frozen at approximately −17°C and thawed prior to feeding. Kelp was added to the pots twice a week after removing uneaten feed and faeces. Dead urchins were noted and removed, but not replaced.

Consumption rate, assimilation rate, and absorption efficiency of urchins held in the various photoperiod treatments were determined at weeks 4 (consumption/assimilation rates) and 12 (consumption/assimilation rates and absorption efficiency) for 15 randomly selected urchins from each treatment. Kelp was wet-weighed prior to feeding. The uneaten kelp and faeces produced over 48 h were collected from each individual urchin, weighed, dried for 72 h at 60°C, and re-weighed at weeks 4 and 12. In addition, at week 12, the dried, uneaten kelp and dried faeces were ashed in a muffle furnace for 4 h at 500°C and re-weighed. The wet weight of each urchin was also determined in both sampling periods. Consumption rate (CR) was calculated using the formula:

$$\mathrm{CR} = (S - E)/U/D$$

where S is the start-wet weight of kelp (g), E, the end-wet weight of kelp (g), U, the urchin wet weight (g), and D, the length of the feeding trial (days). Assimilation rate (AR) was calculated using the formula:

$$\mathrm{AR} = ((S - E) - F)/U/D$$

where F is the faecal wet weight (g). The absorption efficiency (U') at week 12 was calculated for each urchin using the method of Conover (1966):

$$U' = [(F' - E')/(1 - E')(F')] \times 100$$

where F' is the ash-free dry weight: dry weight ratio (fraction of organic matter) in the ingested food, and E' is the same ratio in a representative sample of faeces. We

determined the weight change of kelp in controls without urchins and found no significant weight change after 48 h of immersion (t test, $t_{18} = 0.65$, $P = 0.52$).

Analysis of gonads

At the start of the experiment, 30 randomly selected urchins were dissected to determine the initial gonad state. A random sample of 10–15 urchins (depending on the numbers of mortalities in each treatment) for each photoperiod treatment was dissected at weeks 4, 8, and 12. The gonad index (%) was quantified with the formula: wet gonad weight/whole urchin wet weight×100.

The colour of the gonad from each dissected urchin was quantified using a reflected-light, fibre-optic spectrophotometer (Minolta Chroma Meter CR-100, Konica Minolta Holdings Inc., Tokyo, Japan). Colour was quantitatively measured by breaking it down into three components in a three-dimensional measurement system developed by the Commission Internationale de l'Eclairage (CIE). For the CIE L^* a^* b^* system, the X-axis measures the hue or redness (a^*) and extends from green on the negative side to red on the positive side. The Y-axis measures the chroma or yellowness (b^*) and ranges from blue on the negative side to yellow on the positive side. Any colour can be defined using these two axes. The Z-axis measures the intensity or lightness (L^*) of the colour and extends from black on the negative side to white on the positive. This measurement system allows colour to be accurately and objectively assessed and allows for statistical comparison among samples. The system has been previously used to assess gonad colour in the sea urchins *S. nudus* (Agatsuma 1998) and *S. droebachiensis* (Robinson et al. 2002). After colour measurement, the gonads were then placed in an oven at 60°C until they maintained a constant weight (a minimum of 72 h) and re-weighed to quantify the percent water.

The different stages of gametogenesis (see histology pictures of ovaries and testes in Meidel and Scheibling 1998 and Walker and Lesser 1998) were identified in all gonads sampled (10–15 per treatment) at weeks 0, 4, 8, and 12. The distal end third of one randomly sampled gonad section from each urchin was collected, fixed in Davidson's solution, dehydrated, embedded in paraffin, sectioned (5-µm sections), and stained with haematoxylin and eosin. Histological sections were classified according to the six maturity stages used by Byrne (1990): Stage I, recovering; Stage II, growing; Stage III, premature; Stage IV, mature; Stage V, partly spawned; and Stage VI, spent. For quantitative analysis, three images (a total surface area of 1.74×3 mm^2) of three randomly selected acini per gonad were digitized using a video camera mounted on a compound light microscope (Motic Instruments Inc., BC, Canada). The relative areas occupied by primary gametocytes (oocytes and spermatocytes for females and males, respectively), ova and spermatozoa, nutritive phagocytes, and unoccupied lumen in three randomly selected acini per urchin were evaluated using image analysis software (UTHSCSA Image Tool freeware, http://www.ddsdx.uthscsa.edu/dig/itdesc.html).

Statistical analyses

Two-way ANOVAs were used to compare consumption and assimilation rates, with the fixed factors Photoperiod (Ambient, 0D, 8D, 16D, and 24D) and Time (weeks 4 and 12). Absorption efficiency was examined with a one-way ANOVA with the fixed factor Photoperiod. Temporal patterns in gonad index, percent gonad water, and gonad colour were compared across treatments using two-way ANOVAs with Time (weeks 0, 4, 8, and 12) and Photoperiod (Ambient, 0D, 8D, 16D, and 24D) as fixed factors. Post hoc multiple comparisons among treatment means were carried out using Fisher's LSD tests. The relative areas of nutritive phagocytes (male and female), spermatozoa, and secondary oocytes/ova were compared using two-way ANOVAs with Time and Photoperiod as fixed factors. Because sample sizes varied between sex and time we used a Tukey test with unequal n to carry out the post hoc comparisons (Zar 1999). For each urchin, the mean measurement of relative areas for three acini was considered as a replicate. To balance the analysis, 15 of the 30 urchins sampled at the start of the experiment (week 0) were randomly chosen for each photoperiod treatment. We evaluated normality using Kolmogorov–Smirnov tests and homogeneity of variances using Cochran's tests prior to the analyses. Data were arcsine transformed when necessary to remove significant heterogeneity. When the assumptions were not met, ANOVAs were applied to both the raw and the rank transformed data and the former was chosen when results were the same for the two analyses (Conover 1980).

Results

Mortality rate

The cumulative mortality varied between 0 and 2% (excluding the accidental mortality of five urchins on March 22 due to an interruption of seawater supply) in the different photoperiod treatments. There was no significant difference among treatments with respect to mortality rate (one-way ANOVA, $F_{4,\ 221} = 0.60$, $P = 0.66$).

Consumption rate, assimilation rate, and absorption efficiency

The consumption rate varied significantly with time, but not with photoperiod treatment or with the interaction

Table 1 Results of separate ANOVAs on consumption and assimilation rates, absorption efficiency, gonad index, gonad water, gonad lightness (L^*), gonad hue or redness (a^*), and gonad chroma or yellowness (b^*) during the 12-week experiment

Source of variation	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
	Consumption (g)				Assimilation (g)				Absorption efficiency (%)			
Time	1	0.04	6.58	< 0.05	1	0.00	1.01	0.32				
Photoperiod	4	0.01	2.11	0.08	4	0.01	2.94	0.02	4	1,150.59	0.41	0.81
Time × photoperiod	4	0.01	1.46	0.22	4	0.00	1.09	0.36				
Residual	134	0.01			134	0.00			64	2,920.72		
	Gonad index (%)				Gonad water (%)							
Time	3	669.54	21.70	< 0.001	3	2438.5	173.1	< 0.001				
Photoperiod	4	125.79	4.07	< 0.005	4	25.7	1.8	0.12				
Time × photoperiod	12	14.14	0.46	0.94	12	16.3	1.2	0.32				
Residual	267	30.85			267	14.1						
	Gonad lightness (L^*)				Gonad hue (a^*)				Gonad chroma (b^*)			
Time	3	3,151.00	36.08	< 0.001	3	12.90	0.49	0.68	3	1,332.42	48.41	< 0.001
Photoperiod	4	73.93	0.85	0.50	4	18.78	0.72	0.58	4	124.34	4.52	< 0.001
Time × photoperiod	12	44.33	0.51	0.90	12	21.93	0.85	0.60	12	46.89	1.70	0.07
Residual	267	87.33			267	25.96			267	27.53		

of the two factors (Table 1 and Fig. 1a). Mean consumption rate (±SE) was 0.11 ± 0.01 and 0.14 ± 0.01 g of algae per gram of urchin per 2 days at weeks 4 and 12, respectively (Fig. 1a). Assimilation rate did not vary significantly with time or with the interaction between time and photoperiod treatment, but there was a significant effect of photoperiod treatment due to the relatively high assimilation rate (0.09 ± 0.02 g of algae per gram of urchin per 2 days) in the ambient treatment at week 12 (Table 1 and Fig. 1b). Absorption efficiency was high at week 12 (75.9 ± 6.4%, all treatments pooled) with no significant effect of photoperiod treatment (Table 1 and Fig. 1c).

Analysis of gonads (index, water, and colour)

A two-way ANOVA indicated that gonad index varied significantly with time and photoperiod treatment, but not with the interaction between the two factors (Table 1). The gonad index at the beginning of the experiment was relatively low (8.6 ± 1.3%) and significantly increased over time to reach a maximum of 16.8 ± 1.0% for the 16D treatment (Table 1 and Fig. 2). A one-way ANOVA, examining the gonad index at the end of the 12-week experimental period, revealed a significant difference among photoperiod treatments ($F_{5,\ 77} = 15.67$, $P < 0.001$). Urchins held under a 16D photoperiod had a significantly higher gonad index than urchins held under an 8D photoperiod or those held outside under ambient conditions (Fig. 2). The gonad index for urchins in the 0D with no food treatment remained similar over time (one-way ANOVA, $F_{3,\ 55} = 0.14$, $P = 0.94$, Fig. 2).

Mean percent gonad water varied significantly with time, but not with photoperiod treatment or with the interaction between the two factors (Table 1). Mean percent gonad water increased significantly from week 0 (85.6 ± 0.7%) to week 4 (92.2 ± 0.3%), but then decreased significantly to 78.2 ± 0.3% by the end of the experiment (Fig. 3).

Gonad lightness (L^*) values varied significantly with time, but not with photoperiod treatment or the interaction between the two factors (Table 1). L^* values increased significantly over time (Table 1 and Fig. 4a). Gonad hue or redness (a^*) values were not significantly affected by photoperiod treatment, time, or the interaction between the two factors (Table 1 and Fig. 4b). However, time and photoperiod both had a significant effect on gonad chroma or yellowness (b^*), the highest values occurring at the end of the experiment (Table 1 and Fig. 4c). Urchins held outside under ambient conditions had significantly lower b^* values than urchins from any other photoperiod treatment (Fig. 4c). There were no other significant pairwise comparisons among photoperiod treatments with respect to b^* values (Fig. 4c).

Analysis of gonads (histology)

The frequencies of sea urchins in stages I–VI of the reproductive cycle at each sampling time for the various photoperiod treatments are shown in Fig. 5. At the beginning of the experiment, the majority (57.1%) of urchins were premature and the other urchins were at the growing (39.3%) or recovering (3.6%) stages. Four weeks later, mature urchins (20–30%) were present in all treatments. Some urchins released their gametes as partly spawned and spent stages were observed in the 8D and ambient photoperiod treatments, respectively. Furthermore, the proportion of recovering urchins was two to four times higher than at the beginning of the experiment. At week 8, mature urchins were only present in the ambient treatment and an increase in proportion of urchins in recovering and growing stages revealed a new gametogenic cycle. The proportion of individuals in the recovering stage at week 8 was two times higher in the 0D, 8D, and 24D photoperiod treatments than in the 16D and ambient treatments. At week 12, recovering and growing stages largely dominated. The 8D

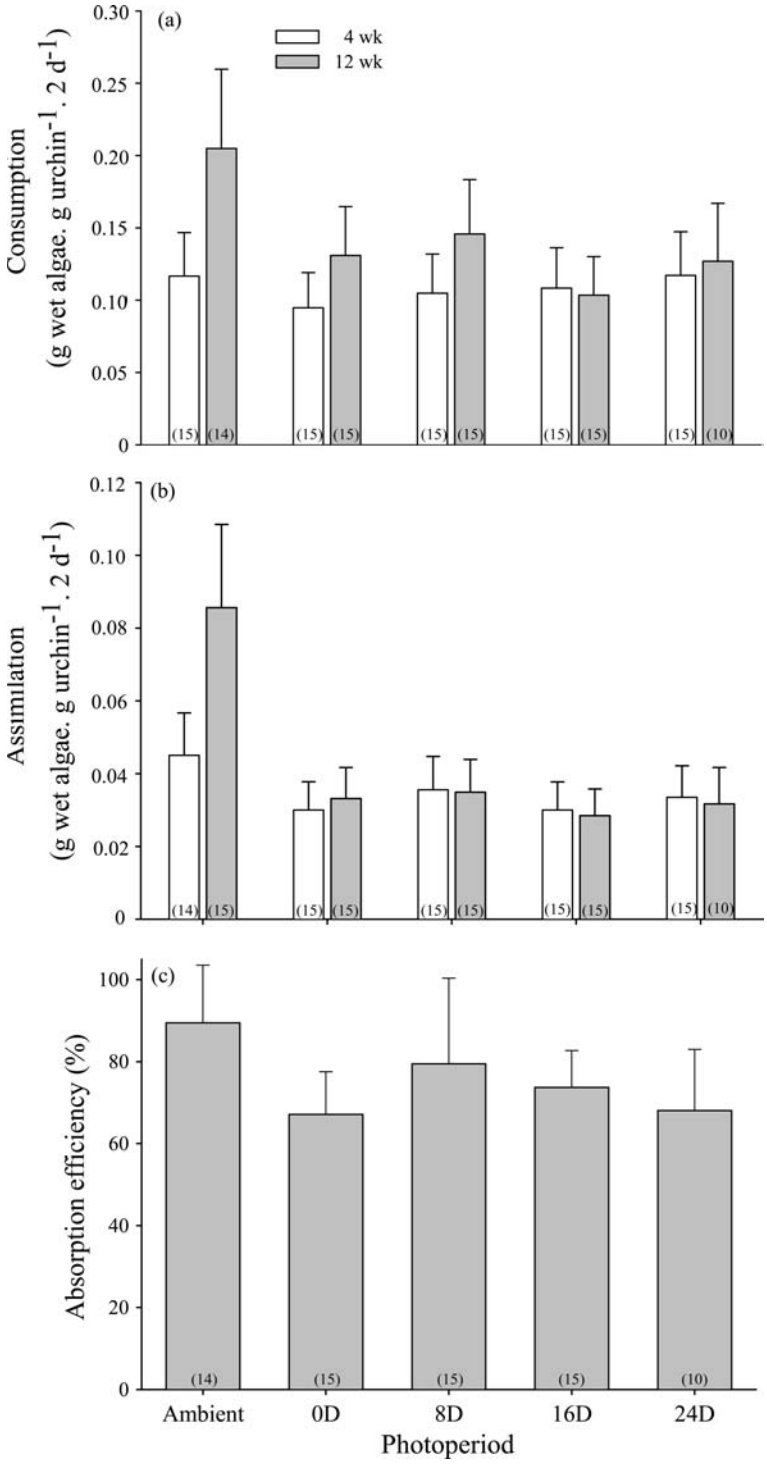

Fig. 1 **a** Mean consumption and **b** mean assimilation rates of sea urchins exposed to five different photoperiods at weeks 4 and 12 of the experiment, and **c** mean absorption efficiency of sea urchins exposed to five different photoperiods at week 12 of the experiment. Photoperiod treatments are: 0D = 0 h dark per day, 8D = 8 h dark per day, 16D = 16 h dark per day, 24D = 24 h dark per day, Ambient = ambient photoperiod with natural light. The feeding measurements were calculated over a period of 2 days. *Error bars* represent standard errors and *numbers in parentheses* indicate the sample size

photoperiod had the highest proportion of recovering urchins and 16D the lowest with 85.7 and 15.3%, respectively. Few premature urchins (6.7–10.0%) were evident in all but the ambient treatment. These differences revealed the beginning of asynchronous gametogenic cycles among the various photoperiod treatments. All six stages of the gametogenic cycle were observed at week 4 and five stages at week 8 (no urchins were in the partly spawned stage) in contrast to three stages (recovering, growing, and premature) at the beginning and the end of the experiment. In contrast to fed urchins, most individuals that were starved under a 0D photoperiod regime had not spawned by the end of the experiment (28.5% had partly spawned) and there was a high proportion of mature urchins (64.3%) with no individuals in the recovering or growing stages at week 12 (Fig. 6).

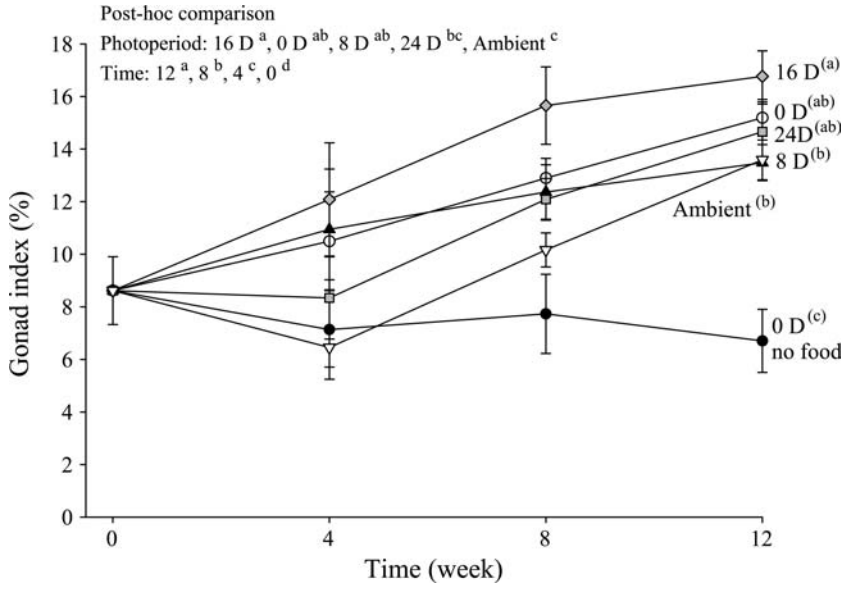

Fig. 2 Mean gonad index (%) of fed sea urchins exposed to five different photoperiods at each sampling time. Photoperiod treatments are: 0D = 0 h dark per day, 8D = 8 h dark per day, 16D = 16 h dark per day, 24D = 24 h dark per day, Ambient = ambient photoperiod with natural light. The treatment "0D no food" represents urchins starved and held under 24 h light per day. Post hoc comparison results (Fisher's LSD test), after two-way ANOVA conducted on all data, indicate significant differences among treatments within the photoperiod and time factors (treatments sharing the same letter are not significantly different, $P > 0.05$). Post hoc results (Fisher's LSD test) are also shown for week 12 data only, following one-way ANOVA, with treatments sharing *the same letter in parentheses* not differing significantly ($P > 0.05$). *Error bars* represent standard errors and $n = 10$–15 urchins

A two-way ANOVA revealed that nutritive phagocyte percent varied significantly with time, photoperiod treatment, and the interaction of the two factors (Table 2). The nutritive phagocytes largely dominated at the end of the experiment in all photoperiod treatments, the proportion increasing significantly from the beginning of the experiment (Table 2 and Fig. 7). The greatest increase in nutritive phagocyte percent was in the 16D photoperiod treatment that had 26.5 ± 9.4 and 69.6 ± 7.3% at weeks 4 and 8, respectively. At week 8, the 8D photoperiod had a significantly higher percent of nutritive phagocytes than the 16D photoperiod, and this difference remained 4 weeks later (Fig. 7).

The percent of spermatozoa varied significantly with time and photoperiod treatment, but not with the interaction of the two factors (Table 2). Male urchins mostly released their spermatozoids between weeks 4 and 8 as an abrupt decrease of spermatozoa occurred in all photoperiod treatments between these two sample

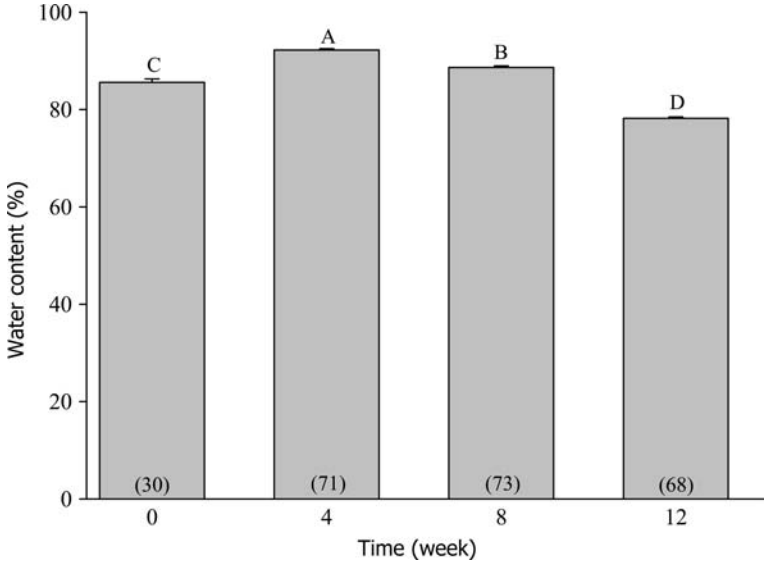

Fig. 3 Mean percent gonad water for all five photoperiod treatments combined at each sampling interval. Post hoc comparison results (Fisher's LSD test) indicate significant differences among weeks (treatments sharing the same letter are not significantly different, $P > 0.05$). *Error bars* represent standard errors and *numbers in parentheses* indicate the sample size

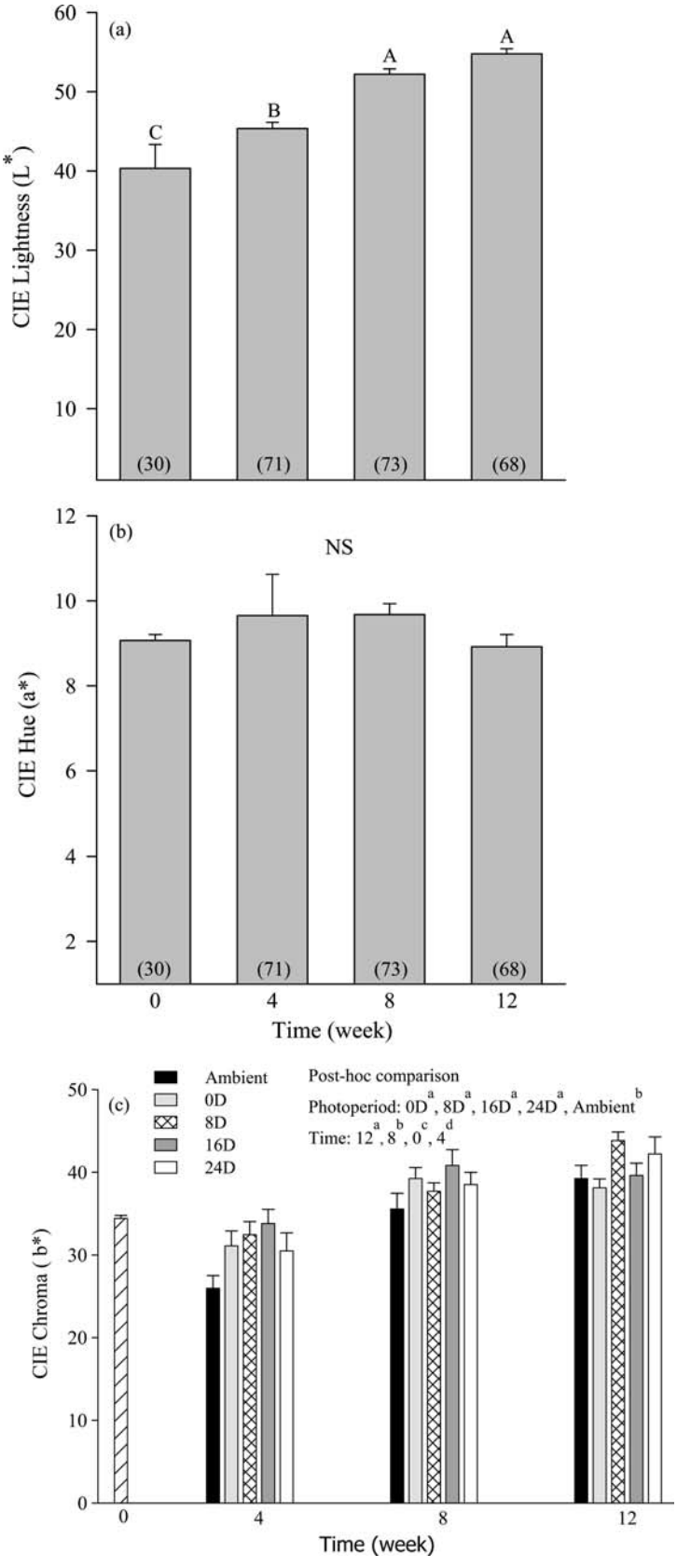

Fig. 4 Mean gonad colour values obtained with a reflected-light spectrophotometer. **a** Mean CIE lightness and **b** mean CIE hue or redness for all five photoperiod treatments combined at each sampling time. Post hoc comparison results (Fisher's LSD test) indicate significant differences among weeks for CIE lightness (treatments sharing the same letter are not significantly different, $P > 0.05$). NS denotes non-significance ($P > 0.05$) among treatments for CIE hue. *Error bars* represent standard errors and *numbers in parentheses* indicate the sample size. **c** Mean CIE chroma or yellowness of the five photoperiod treatments at each sampling time. Post hoc comparison results (Fisher's LSD test) indicate significant differences among treatments within the photoperiod and time factors (treatments sharing the same letter are not significantly different, $P > 0.05$). *Error bars* represent standard errors and $n = 10$–15 urchins. For all three figures, photoperiod treatments are: 0D = 0 h dark per day, 8D = 8 h dark per day, 16D = 16 h dark per day, 24D = 24 h dark per day, Ambient = ambient photoperiod with natural light

periods (Fig. 8). The proportion of spermatozoa thereafter remained low until the end of the experiment. The proportion of spermatozoa was the highest in the 16D photoperiod treatment, but it was only significantly different from the ambient treatment (Table 2 and Fig. 8).

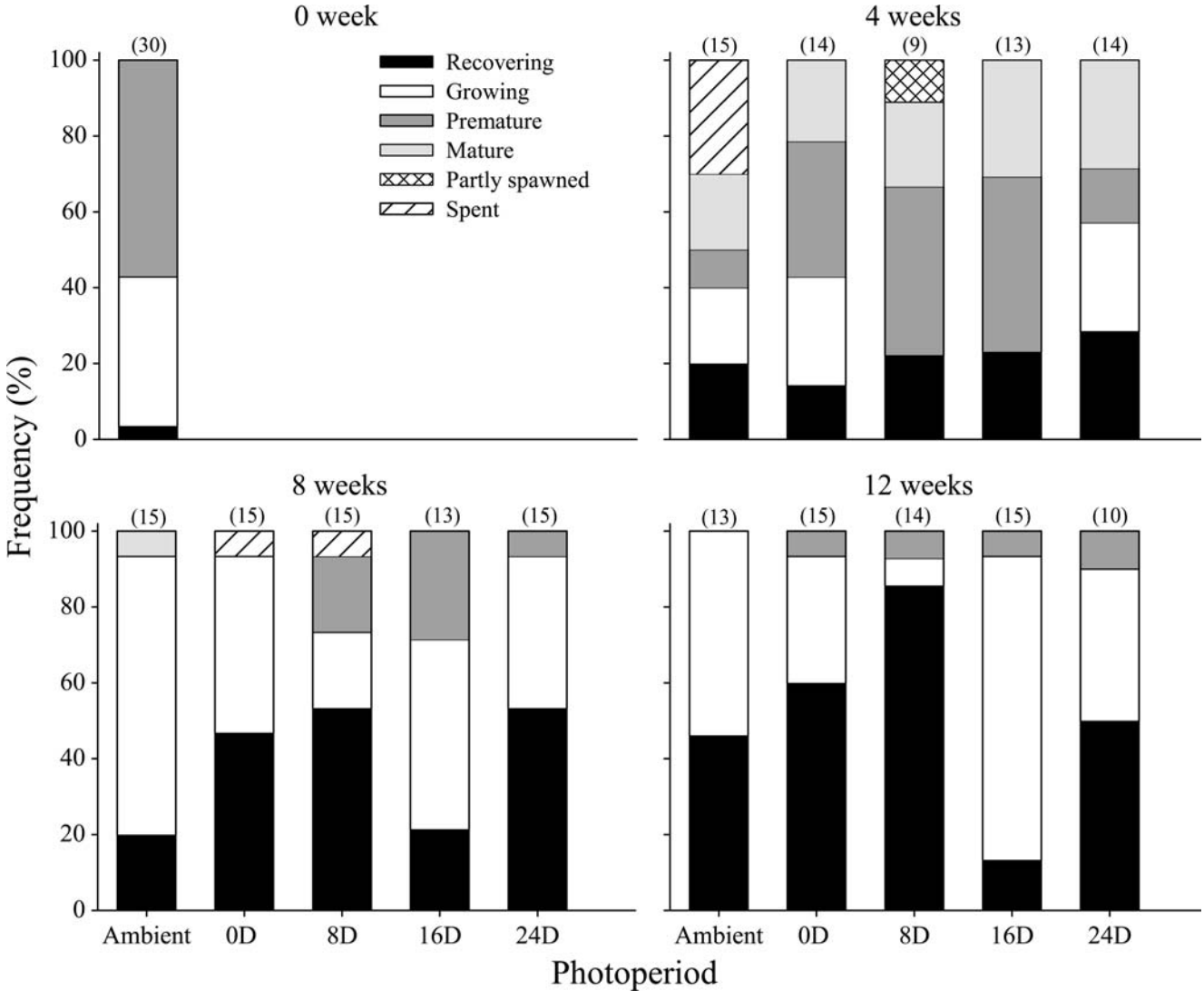

Fig. 5 Mean frequency (%) of fed sea urchins in stages I–VI in the reproductive cycle at each sampling time for the five photoperiod treatments. Photoperiod treatments are: 0D = 0 h dark per day, 8D = 8 h dark per day, 16D = 16 h dark per day, 24D = 24 h dark per day, Ambient = ambient photoperiod with natural light. *Numbers in parentheses* indicate the sample size

Table 2 Results of separate two-way ANOVAs on percent of gonad area occupied by nutritive phagocytes, spermatozoa, and oocytes II and ova during the 12-week experiment, and results of post hoc comparisons (Tukey tests with unequal *n*) of the effects of photoperiod and time on nutritive phagocytes

Source of variation	*df*	MS	*F*	*P*	Source of variation	*df*	MS	*F*	*P*
Nutritive phagocytes					Spermatozoa				
Time	3	16059.23	28.35	< 0.001	Time	3	3217.83	20.99	< 0.001
Photoperiod	4	2089.25	3.69	< 0.01	Photoperiod	4	867.41	5.66	< 0.001
Time × photoperiod	12	1033.01	1.82	< 0.05	Time × photoperiod	12	171.00	1.12	0.36
Residual	256	566.54			Residual	76	153.28		
Post hoc comparisons					Oocytes II and ova				
Photoperiod	Time				Time	3	13827.86	17.60	< 0.001
Ambient: 0^{b} 4^{ab} 8^{a} 12^{a}	4 week: $16D^{b}$ $8D^{ab}$ $0D^{ab}$ $24D^{a}$ $Ambient^{a}$				Photoperiod	4	279.84	0.36	0.84
0D: 0^{c} 4^{bc} 8^{ab} 12^{a}	8 week: $16D^{b}$ $Ambient^{ab}$ $24D^{a}$ $8D^{a}$ $0D^{a}$				Time × photoperiod	12	973.33	1.24	0.26
8D: 0^{b} 4^{b} 8^{ab} 12^{a}	12 week: $16D^{b}$ $24D^{ab}$ $0D^{ab}$ $Ambient^{ab}$ $8D^{a}$				Residual	156	785.54		
16D: 4^{b} 0^{ab} 8^{a} 12^{a}									
24D: 0^{b} 4^{ab} 8^{ab} 12^{a}									

Treatments with the same letter are not significantly different ($P > 0.05$)

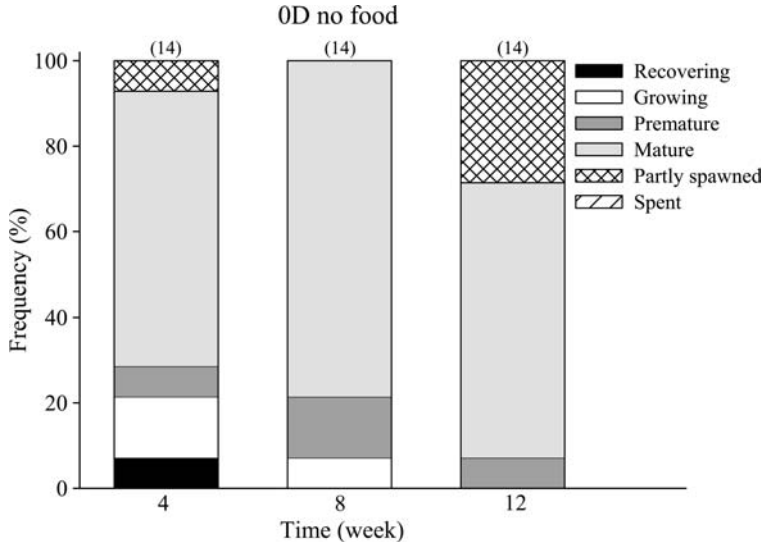

Fig. 6 Mean frequency (%) of starved sea urchins held under a 24 h light photoperiod (0D) in stages I–VI in the reproductive cycle at each sampling time. *Numbers in parentheses* indicate the sample size

The percent of secondary oocytes and ova varied significantly with time, but not with photoperiod treatment or with the interaction between the two factors (Table 2). Similarly to the males, female urchins spawned between weeks 4 and 8 (Fig. 9). The proportion of secondary oocytes and ova did not differ between the beginning of the experiment and week 4, but the release of gametes after that week resulted in a significant decrease of mature gametes between weeks 4 and 8 (Table 2 and Fig. 9). There were no significant differences among photoperiod treatments in the percent of secondary oocytes or ova during the 12-week experiment.

Discussion

Except for elevated levels at week 12 in individuals held outside under ambient lighting, photoperiod regime had little effect on consumption rate, assimilation rate, or absorption efficiency of green sea urchins. These results are similar to the findings of Pearse et al. (1986a) who reported no significant difference in feeding rates of juvenile purple sea urchins, *Strongylocentrotus purpuratus*, held under two different photoperiod regimes: (1) ambient photoperiod and (2) 6 months out of phase with the ambient photoperiod. In addition, Shpigel et al. (2004) found no effect of photoperiod regime (i.e. 8, 16, and 24 h dark and ambient photoperiod) on feeding rate of adult *Paracentrotus lividus*. It is apparent, from these studies and the present one, that energy intake and utilization in these echinoids are not significantly affected by the number of daylight hours. Thus, any potential differences in gonad development among the various photoperiod regimes in the present study cannot be ascribed to significant variation among the treatments with regards to feeding, assimilation, or absorption.

The gonad index at the beginning of the experiment was 8.6% and significantly increased over time to reach a minimum of 13.5% in the 8D treatment and a maximum of 16.8% in the 16D treatment. Thus, increase in

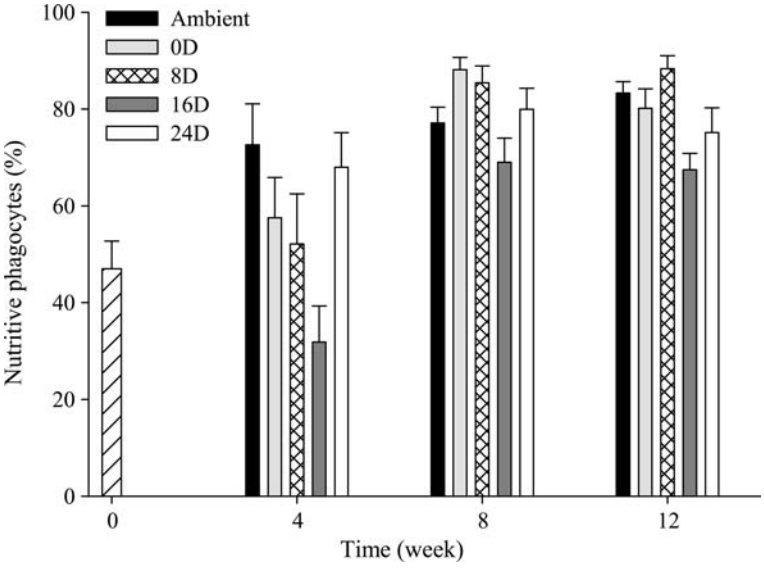

Fig. 7 Mean percent of gonad area occupied by nutritive phagocytes (male and female) at each sampling time for the five photoperiod treatments. Photoperiod treatments are: 0D = 0 h dark per day, 8D = 8 h dark per day, 16D = 16 h dark per day, 24D = 24 h dark per day, Ambient = ambient photoperiod with natural light. Post hoc comparison results (Tukey test) indicate significant differences among treatments within the photoperiod and time factors and are given in Table 2 (treatments sharing the same letter are not significantly different, $P > 0.05$). *Error bars* represent standard errors and $n = 9$–15

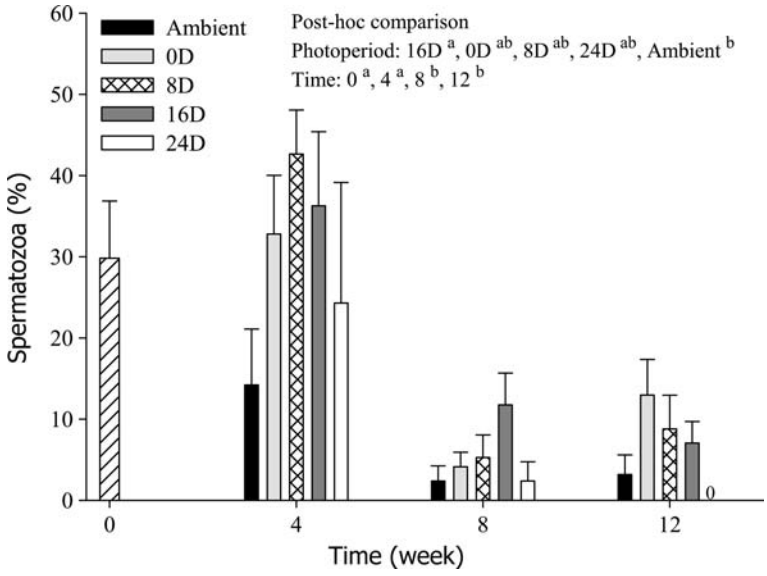

Fig. 8 Mean percent of gonad area occupied by spermatozoa at each sampling time for the five photoperiod treatments. Photoperiod treatments are: 0D = 0 h dark per day, 8D = 8 h dark per day, 16D = 16 h dark per day, 24D = 24 h dark per day, Ambient = ambient photoperiod with natural light. Post hoc comparison results (Tukey test) indicate significant differences among treatments within the photoperiod and time factors (treatments sharing the same letter are not significantly different, $P > 0.05$). *Error bars* represent standard errors and $n = 3$–9 male urchins

gonad index per week ranged from 0.4 to 0.7% week^{-1}. These are typical rates of gonad growth in green sea urchins held in captivity and fed kelp (Pearce et al. 2002a, b, c). While there were slight declines in gonad indices between weeks 0 and 4 for the 24D and ambient treatments, the general trend in all photic regimes receiving food was an increase in gonad index at each sampling period. This trend occurred despite the fact that many individuals spawned between weeks 4 and 8. A number of studies with *S. droebachiensis* have shown that gonad growth and gamete production are positively related to both food quantity (Vadas 1977; Thompson 1983, 1984; Keats et al. 1984; Hooper et al. 1996; Minor and Scheibling 1997; Hagen 1998; Meidel and Scheibling 1998, 1999; Garrido and Barber 2001; Pearce et al. 2002c) and quality (Vadas 1977; Larson et al. 1980; Keats et al. 1984; Cuthbert et al. 1995; de Jong-Westman et al. 1995; Hooper et al. 1996; Lemire and Himmelman 1996; Russell 1998; Meidel and Scheibling 1999; Vadas et al. 2000; Scheibling and Anthony 2001; Pearce et al. 2002a, b, 2003, 2004) and ample food supplies may ensure enough energy to promote overall gonad growth even during times of spawning. In the present experiment, urchins that were starved had an overall decrease in gonad index over the 12-week experiment, indicating that they did not have sufficient energy for gonad production.

There is little evidence to support the contention that food abundance alone is the sole extraneous factor responsible for initiating or timing gametogenesis in the majority of echinoids (Gonor 1973; Pearse 1981; Pearse et al. 1986a). Since food levels can be quite variable

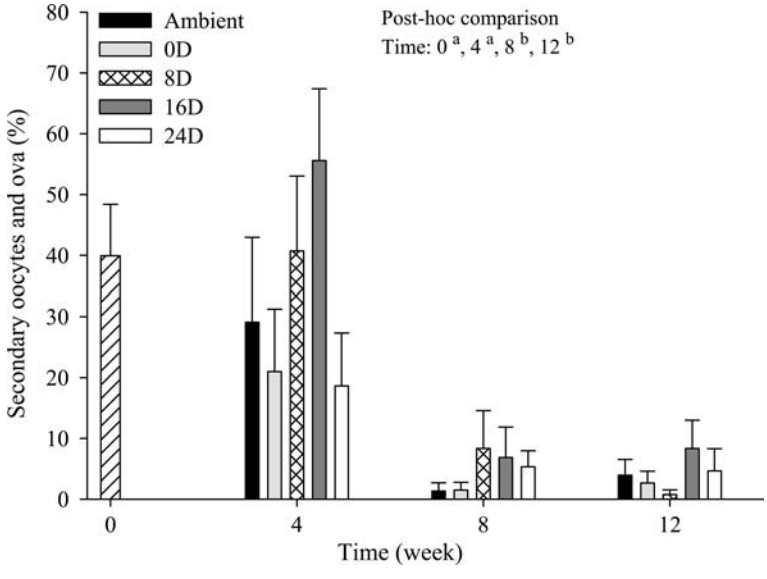

Fig. 9 Mean percent of gonad area occupied by secondary oocytes and ova at each sampling time for the five photoperiod treatments. Photoperiod treatments are: 0D = 0 h dark per day, 8D = 8 h dark per day, 16D = 16 h dark per day, 24D = 24 h dark per day, Ambient = ambient photoperiod with natural light. Post hoc comparison results (Tukey test) indicate significant differences among weeks (treatments sharing the same letter are not significantly different, $P > 0.05$). *Error bars* represent standard errors and $n = 6$–11 female urchins

among years and locations, food abundance is an unlikely candidate to entrain reproductive cycles. However, Pearse (1969a) suggested that fluctuations in food may be important in regulating reproductive periodicities of the echinoids *Prionocidaris baculosa* and *Lovenia elongata* in the Gulf of Suez, but hypothesized that photoperiod and/or temperature may be indirectly associated with the synchronization of reproduction in these species through their direct affects on food levels and/or feeding/ metabolic rates.

Environmental factors that are relatively more stable on an inter-annual basis may be required in order to entrain yearly reproductive cycles. In echinoids, both photoperiod (Boolootian 1963; Holland 1967; Gonor 1973; Pearse et al. 1986a; Bay-Schmith and Pearse 1987; Byrne 1990; McClintock and Watts 1990; King et al. 1994; Hagen 1997; Walker and Lesser 1998; Kelly 2001; Shpigel et al. 2004) and temperature (Chatlynne 1969; Khotimchenko 1982; Yamamoto et al. 1988; Sakairi et al. 1989) have been linked with the initiation of gametogenesis. In species thought to be governed by photoperiod, there are those where gametogenesis is controlled by short day-lengths such as *S. purpuratus* (Pearse et al. 1986a; Bay-Schmith and Pearse 1987), *Eucidaris tribuloides* (McClintock and Watts 1990), *S. droebachiensis* (Walker and Lesser 1998), *Paracentrotus lividus* (Shpigel et al. 2004) and others that are considered long-day species such as *Psammechinus miliaris* (Kelly 2001).

It is obvious from the results of the current study that once gametogenesis is initiated, spawning cannot be halted by photoperiod regulation. It has been shown with other echinoderm species that individuals collected from the wild and placed under experimental conditions can retain their internal reproductive calendars for long periods of time (Leahy et al. 1981; Pearse et al. 1986b). Results from the present study suggest, however, that the rate at which urchins progress through the various developmental stages of the gametogenic cycle can be affected by photoperiod regime. By the end of the 12-week experiment, urchins in all five fed photoperiod treatments had completely spawned out, but the proportion of individuals in the recovering versus growing stages was markedly different. Urchins in the 16D treatment had by far the greatest percentage of individuals in the growing stage.

Interestingly, the photoperiod treatment that was most similar in gametogenic development to 16D at the end of the experiment was the ambient photoperiod which was also a short-day treatment (range: 15.25 h of darkness at the beginning of the experiment to 10.50 h of darkness at the end of the experiment). While similar to the 16D photoperiod in terms of reproductive stage of development, the ambient treatment had a significantly lower gonad index than the 16D treatment, despite the following: (1) urchins in both treatments were fed the same quality and quantity of food (kelp ad libitum), (2) urchins in both treatments were held at the same temperature and in the same type of containment system, and (3) urchins in the ambient treatment consumed and assimilated more food at week 12 than those in the 16D photoperiod. The only obvious difference between the two treatments was in the quality and intensity of light, ambient urchins being exposed to natural sunlight and 16D urchins to fluorescent light. There is the possibility that the artificial lighting in the 16D treatment resulted in the development of thicker or more nutritious microbial films on the walls of the containment systems than in the ambient photoperiod treatment and that urchin grazing of these films led to a higher gonad index in the 16D photoperiod. However, the 8D treatment also had a significantly lower gonad index than the 16D treatment at the end of the experiment, and both treatments involved exposure to fluorescent light. Results from other studies examining the effect of photoperiod on urchin gonad indices indicate that short day-lengths may favour increased gonad production, but the results are variable. Spirlet et al. (2000) found that gonad production of *Paracentrotus lividus* was significantly higher under short day-lengths (9L:15D) than under long day-lengths (17L:7D), but at only one of four temperatures tested. McClintock and Watts (1990) reported that gonad indices of *Eucidaris tribuloides* were higher in a fixed short-day treatment (9L:15D) than in a fixed long-day treatment (15L:9D) for the last three sampling dates of a 1-year long experiment (significantly higher at two of these dates). Other studies examining the effects of photoperiod on echinoid gonad development, however, have reported no significant effect of photic regime on gonad indices [Pearse et al. (1986a) for *S. purpuratus*, Kelly (2001) for *Psammechinus miliaris*, Shpigel et al. (2004) for *Paracentrotus lividus*]. Currently, it is unclear what factors in the present study may have contributed to differences in gonad indices among the various photoperiod treatments. The photosensitivity of echinoids is well known (Millott 1975), but intriguing behavioural responses such as increased activity and decreased covering behaviour have been observed under artificial light (Verling et al. 2002). A better understanding of the effects of different sources and intensities of artificial light is required to further improve the control of gametogenesis and gonad quality.

Since the commercial urchin market prefers gonads in the growing or premature stages—where the gonads are generally large and have a high percentage of nutritive phagocytes and low occurrence of mature gametes—land-based urchin aquaculture operations may optimize production of suitable gonads for market through the use of short day-lengths.

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