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# Suspension of annual gametogenesis in North American green sea urchins (*Strongylocentrotus droebachiensis*) experiencing invariant photoperiod—Applications for land-based aquaculture

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## Abstract

Sea urchin fisheries are valuable commercial resources in the United States with processed gonads sold in Japanese and American markets and maximum US sales of \$150M US dollars in 1996. Wild populations of sea urchins on all coasts of the US have been dramatically over-fished. Aquaculture of sea urchins in land-based facilities can help restore commercial populations and preserve this ecologically important herbivore. In this study, we used invariant summer photoperiod to prevent gametogenesis in the North American green sea urchin (*Strongylocentrotus droebachiensis*) maintained in a land-based aquaculture system and provided a commercially available formulated feed that promotes maximum growth of intra-gonadal somatic nutrient storage cells called nutritive phagocytes. Results were compared with individuals fed the same formulated feed under ambient photoperiod in cages in the ocean. Monthly samples of gonads from both treatments were evaluated for gonad index, volume fractions of cellular constituents of the germinal epithelium, oocyte diameters and taste. Over the 5 months of this study, gonad indices increased significantly ( $p < 0.001$ ) in both treatments from  $4.8\% \pm 0.9$  (all values  $\pm$  SE) initially to  $20.5\% \pm 2.1$  under invariant and  $23.2\% \pm 1.4$  under ambient photoperiod with no significant difference between treatments ( $p = 0.55$ ). Volume fractions of nutritive phagocytes increased to  $80.3\% \pm 5.9$  (initial  $37.9\% \pm 7.1$ ) in males and  $71.0\% \pm 6.7$  (initial  $10.3\% \pm 4.0$ ) in females ( $p < 0.001$ ) only under invariant photoperiod. Nutritive phagocyte lengths increased under both photoperiod treatments, but the volume fraction containing nutrients was higher under invariant photoperiod. Volume fractions of gonial/gametogenic cells increased significantly ( $p < 0.001$ ) only under ambient photoperiod from  $20.4\% \pm 5.5$  to  $37.8\% \pm 1.8$  in males and  $0\%$  to  $22.6\% \pm 3.6$  in females. The volume fraction of residual oocytes from last year's oogenesis increased under invariant photoperiod while that of both residual and new oocytes increased under ambient photoperiod. Residual oocyte diameters increased from  $56.2 \mu\text{m} \pm 2.2$  initially to  $93.5 \mu\text{m} \pm 3.7$  under invariant and those of residual and new oocytes to  $126.0 \mu\text{m} \pm 7.3$  under ambient photoperiod. Invariant photoperiod yields gonads in both sexes of *S. droebachiensis* that do not initiate fall gametogenesis but attain large size as their nutritive phagocytes grow substantially in size. A Canadian study

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of wild-collected *S. droebachiensis* indicated that gonads taste best when they contain pre-dominantly nutritive phagocytes and not copious gametes, however gonad taste in our study was unsatisfactory suggesting that the only commercially available sea urchin diet requires modification to support commercial development of land-based aquaculture.

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*Keywords:* *Strongylocentrotus droebachiensis*; Invariant photoperiod; Gametogenesis; Nutritive phagocytes; Aquaculture; Formulated feed

## 1. Introduction

Since the late 1980s, the Maine fishery for the green sea urchin, *Strongylocentrotus droebachiensis*, has become the largest urchin fishery in the United States (approximately \$36M annually, Maine Department of Marine Resources, 1994–96). Unregulated harvesting has drastically depleted once abundant natural populations resulting in significant economical and ecological impacts (Chen and Hunter, 2003; Taylor, 2004). In addition to over-fishing, other circumstances have contributed to a decline in the value of sea urchin landings. One is the poor quality of a large percentage of gonads available in the remaining wild sea urchins (personal communication, Mr. Atchan Tamaki, President of I.S.F. Trading, Portland Maine). Other problems are related to the biology of sea urchin gametogenesis. These include: 1) the short annual window of time when gonad quality is maximal and 2) the disparity in timing between maximum gonad quality and peak product value in the commercial markets. This paper proposes a method for suspending gametogenesis in the *S. droebachiensis* that allows land-based aquaculturists to extend the time during which sea urchin gonad quality is highest.

Gametogenesis and intra-gonadal nutrient storage and utilization are linked processes in sea urchin reproduction (Walker et al., 2006). Initial increase in size of sea urchin gonads results as somatic cells within the germinal epithelium, the nutritive phagocytes (NPs), store extensive nutrient reserves before gametogenesis commences. In *S. droebachiensis* (Walker and Lesser, 1998) shorter day-lengths in the fall are closely correlated with initiation of gametogenesis. NPs simultaneously mobilize nutrients as spermatogonia and oogonia begin extensive mitosis. When intra-gonadal nutrient reserves are transferred from nutritive phagocytes to gametes during gametogenesis, the commercial quality of gonads from *S. droebachiensis* progressively decreases (Komata et al., 1962; Hirano et al., 1978; Lee and Haard, 1982; Unuma, 2002; Unuma et al., 2003; Böttger et al., 2004; Reunov et al., 2004; Dumont et al., 2006). High quality sea urchin

gonads are distinguished by large size and by the quality of a number of sensory parameters (taste, color, texture and firmness). Annually, such high quality gonads exist in *S. droebachiensis* between September and early January. During this time period (corresponding to the late Pre-Gametogenesis and NP Renewal and early Gametogenesis and NP Utilization stages; Walker et al., 2005, 2006), ovaries and testes from natural populations are preferred by Japanese consumers and contain fewer gametes relative to NP, are large, bright orange or yellow, sweet tasting and firm (Lee and Haard, 1982; Unuma, 2001, 2003; Walker et al., 2005, 2006; Dumont et al., 2006).

It has been suggested that successful manipulation of gametogenesis in sea urchins will be vitally important for the future of the New England sea urchin fishery and for development of land-based aquaculture (Walker et al., 2006). Several studies have addressed the possibility that photoperiod can be manipulated at various times of the year to suspend gametogenesis in order to duplicate and enhance the desirable size and sensory qualities present in wild-collected gonads of *S. droebachiensis* late in the Pre-Gametogenesis and NP Renewal and early Gametogenesis and NP Utilization stages (Walker et al., 2005, 2006). Following spring spawning, Walker and Lesser (1998) exposed *S. droebachiensis* to pre-mature summer photoperiod that then progressed under the control of an astronomic clock. This resulted in a second peak in gonad size during the summer (representing out-of-phase December photoperiod). This changing photoperiod regime provided the photoperiod cue (in out-of-phase October) and gametogenesis was initiated in these gonads. However, prior to this, large gonads did contain predominantly NPs. No sensory analysis was performed for this study. Dumont et al. (2006) extended these observations and demonstrated that once *S. droebachiensis* have experienced the photoperiod cue (= the autumnal equinox) they will complete gametogenesis and spawn under all photoperiod regimes tested; sensory analysis was made in this study for color only. In the current study, we have manipulated photoperiod during the remaining unevaluated period of the year. *S.*

Table 1  
Components of the Wenger formulated feed used in this study

Component	Percentage (%)
Plant meal (corn, wheat, soybean)	60.0
Marine meal (fish, kelp, squid, krill)	25.6
Carotenoid	0.8
Vitamin pre-mix	0.3
Antioxidant	0.2
Cholesterol	0.3
Oil (plant and marine animal)	1.0
General pre-mix	3.4
Phospholipid (soy lecithin)	1.0
Antifungal (glycerin, sodium sorbate)	7.6

*droebachiensis* were collected in the summer, maintained under invariant summer photoperiod (July) and fed a commercially available formulated sea urchin feed. Gonads from these urchins were compared with those collected at the same time from urchins suspended in cages in an estuarine lease site in water of the same temperature and salinity and fed the same formulated feed but experiencing ambient photoperiod. In the absence of changing fall photoperiod, sea urchins should suspend gametogenesis and produce large gonads that contain pre-dominantly nutritive phagocytes. The sensory qualities of these gonads will then depend upon the formulated feed they receive during the experimental period. In this study, we utilized a commercially available formulated sea urchin feed (Wenger Interna-

tional, Inc.) and a sea urchin buyer in Maine to evaluate size, taste, color and firmness of the gonads resulting from both treatments.

## 2. Materials and methods

### 2.1. Collection and Maintenance of *S. droebachiensis*

Seven hundred green sea urchins (*S. droebachiensis*) between 40 and 65 mm in test diameter were collected by hand at the Peacock Cannery in Lubec Maine (44°51.603' N, 66°58.904'W) at the lowest tide in July 2004. They were transported to the flowing seawater laboratory at the Darling Center of the University of Maine and acclimated without feeding for 2 weeks under ambient photoperiod (using artificial daylight) in a modular aquaculture system (Devin, 2001). This system consists of fiberglass troughs with slanted sides (designed to account for the morphology and behavior of adult sea urchins), with a drainage channel along the length of each trough and a drainage sump at the discharge of each of these troughs; each trough is separately supplied with seawater. Depending on the diameter of the sea urchins, each of the nine troughs in this system can maintain 100–300 individuals.

Following acclimation, three hundred sea urchins were randomly distributed across three troughs of the modular system for the invariant photoperiod treatment. Tanks were equipped with fluorescent lights (General Electric

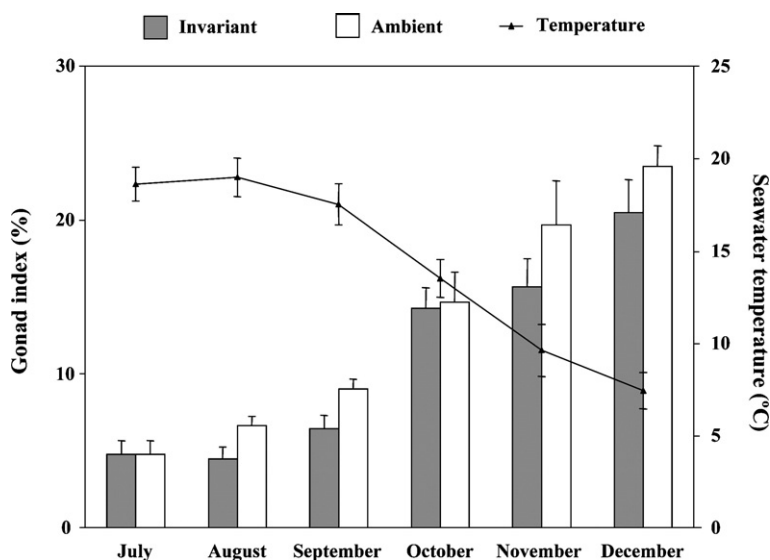


Fig. 1. Mean gonad indices (%)±SE of *Strongylocentrotus droebachiensis* maintained under invariant (July) and ambient photoperiods over five months (July–December, 2004). Mean monthly temperatures (°C)±SE, obtained from measurements of Damariscotta River provided and by the Darling Marine Center, are also indicated.

F40D Daylight; irradiance of  $\sim 50 \mu\text{mol quanta m}^{-2}$ ) controlled by astronomic time switches (BRK Electronics, Model TLA24, Aurora, Illinois). These animals were maintained for 5 months at photoperiod corresponding to a calendar date in mid-July (using sunrise and sunset values in the 2004 Farmers' Almanac). Another three hundred sea urchins were distributed between five cages (60 animals/cage; AquaPurses [ $L \times W \times H = 80 \times 36 \times 20 \text{ cm}^3$ , mesh size  $2 \times 1.5 \text{ cm}^2$ , black plastic] designed for bivalve culture, ToolTech, Australia) that were suspended alongside each other on a line at a lease site of the University of Maine's Darling Marine Center in the Damariscotta river estuary (saline river at the site is 28–32‰,  $43^\circ 56.595' \text{ N}$ ,  $69^\circ 34.529' \text{ W}$ ), which is also the source for the running seawater in our experimental tanks. These sea urchins constituted the ambient photoperiod treatment. Individuals in both treatments were fed a commercially available sea urchin pelletized formulated feed at 10% of their body weight every 48 h for 5 months.

The diet was delivered by placing it throughout the troughs in the invariant photoperiod treatment. Cages used for ambient photoperiod were removed from the water and two feed bags ( $0.75 \text{ cm}^2$  mesh size, pellet size  $0.8 \text{ cm}^3$ ) containing feed pellets added to the purses, while old feed bags were removed. This formulated feed is available through Wenger International, Inc. (Table 1; Kansas City, Kansas, USA) (Lawrence et al., 1997; Klinger et al., 1998; Watts et al., 1998; Walker and Lesser, 1998; Kearns, 2004). Encrusting organisms (e.g., *Membranipora* sp.) and drift algae were also available to sea urchins in the cages.

## 2.2. Monthly sampling

Ten sea urchins were sacrificed at the start of these studies and subsequent monthly samples were taken of ten sea urchins each from the trough systems (invariant photoperiod—three individuals from two and four from

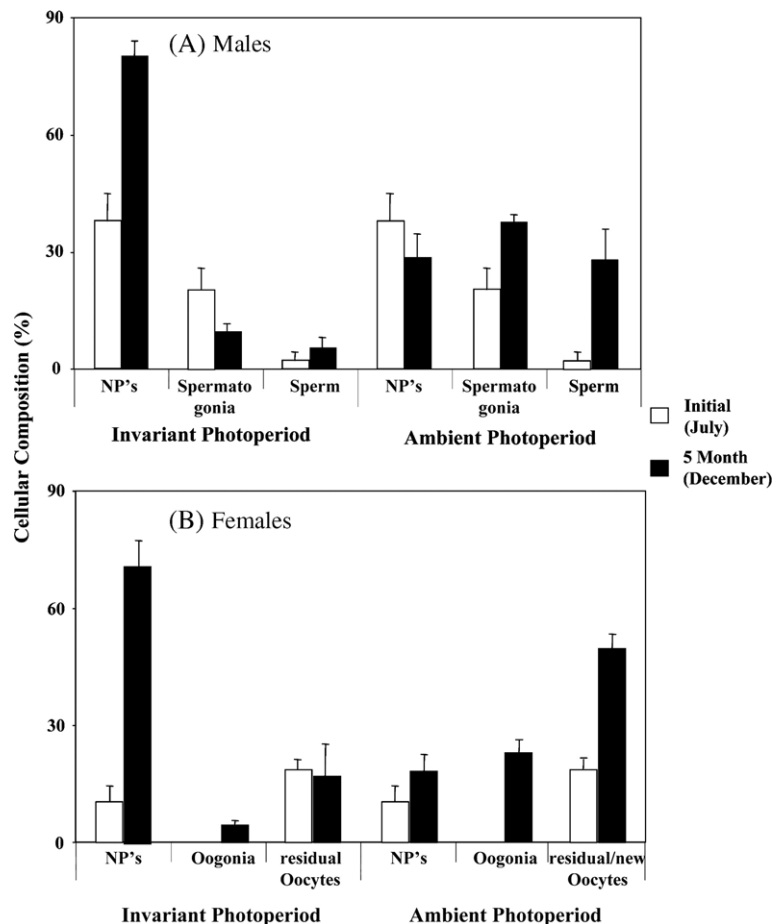


Fig. 2. Mean volume fractions (%)  $\pm$  SE of the major cellular constituents of gonads from (A) male and (B) female *Strongylocentrotus droebachiensis* maintained under invariant (July) and ambient photoperiods for 5 months (July–December, 2004).

one of the troughs) and cages (ambient photoperiod—two individuals from each cage). For each collection, test height and diameter of each urchin was measured to the nearest 0.1 mm with vernier calipers and the total weight to the nearest 0.1 mg. The gonads were removed, blotted dry, their wet weights determined and a gonad index was calculated as a percent of the total wet weight of the urchin. A central portion of the gonad from each urchin was prepared for histological and stereological analysis.

### 2.3. Histology and stereology

In order to determine gametogenic stage (Walker et al., 2005), gonads from each sea urchin were fixed for 2 h in primary fixative (3% glutaraldehyde in 0.2 M sodium cacodylate buffer) followed by rinses in cacodylate buffers with decreasing salt concentrations (Walker, 1980). Post fixation in 1% osmium tetroxide in 0.2 M cacodylate buffer was followed by dehydration

in an alcohol series. Tissues were finally embedded in 50% Epon–Araldite, 50% DDSA overnight in a 60 °C drying oven and sectioned at 1–5  $\mu\text{m}$  on a Reichert OM U3 ultramicrotome and stained with buffered 0.2% azure B in 1%  $\text{NaHCO}_3$  at 60 °C. All sections were observed and photographed on a Zeiss Axioplan-2 light microscope at 20–60 $\times$ .

To obtain monthly volume fractions ( $V_v$ ) for gonial cells, nutritive phagocytes and residual and new gametes, the relative proportion of different cell types within the germinal epithelium of each gonad was evaluated by stereology after over-laying photographs of sections with a grid (Elias and Hyde, 1980; Walker and Lesser, 1998; Harrington et al., in press).

Nutritive phagocyte sizes (= length in  $\mu\text{m}$ ) were determined for 20 nutritive phagocytes/sea urchin using a Zeiss Axioplan-2 light microscope. Measurements of NPs included growing primary oocytes contained within NP incubation chambers (Walker et al., 2005). These

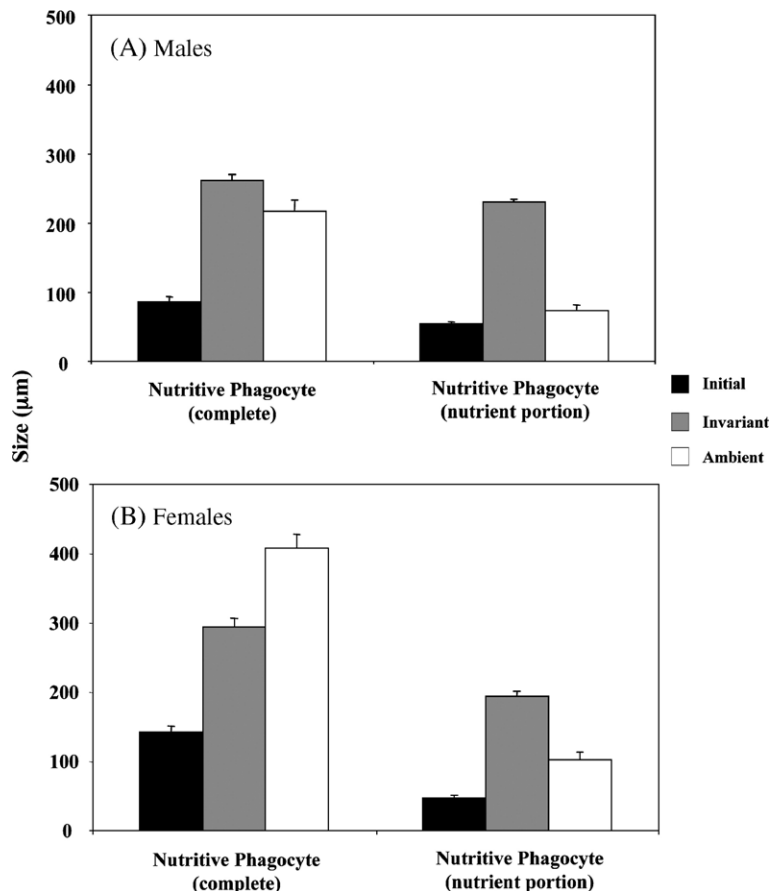


Fig. 3. Mean volume fractions of the nutritive phagocytes ( $\mu\text{m}$ ) $\pm$ SE of *Strongylocentrotus droebachiensis* were evaluated separately for (A) males and (B) females at time zero and after culture for 5 months under invariant and ambient photoperiods. The measurements were calculated as the total size of the nutritive phagocytes (including gonial cells and developing primary oocytes) and the nutrient portion of the nutritive phagocytes alone, using results previously determined through stereology.

measurements were then adjusted to reflect the nutrient portion of the nutritive phagocyte by subtracting spermatocytes and spermatogonia in males and oocytes and oogonia in females.

Primary oocyte long diameters were measured where the nucleolus was evident for 50 primary oocytes/individual at time zero (July) and at the termination of the study (December) for both photoperiod treatments. Measurements were performed at 20 $\times$  using a Zeiss Axioplan-2 light microscope.

#### 2.4. Statistical analysis

Gonad indices were analyzed using descriptive statistics followed by a Two-Way ANOVA, stereological measurements, nutritive phagocyte sizes and oocyte diameters were evaluated statistically using descriptive statistics and a One-Way ANOVA on Ranks followed by a Tukey pairwise comparison (SigmaStat, Systat Software Inc., Point Richmond, CA). All statistical analyses were preceded by assessments of the assumption of normality (Kolmogorov–Smirnov Test) and homoscedasticity (Spearman Rank Correlation).

### 3. Results

#### 3.1. Gonad indices

Gonad indices increased significantly ( $p < 0.001$ ,  $F = 46.37$ , degrees of freedom = 5) over 5 months for both photoperiod treatments. Urchins maintained under invariant photoperiod reached a gonad index of  $20.5\% \pm 2.1$ , while individuals maintained at ambient photoperiod increased to  $23.5\% \pm 1.3$  from the initial value ( $4.8\% \pm 0.9$ ) (Fig. 1). There was no significant difference between individuals maintained in invariant and ambient photoperiod treatments across all dates ( $p = 0.55$ ,  $F = 0.93$ , degrees of freedom = 19).

#### 3.2. Stereology, nutritive phagocyte length and oocyte diameter

Under invariant photoperiod, there was a significant increase ( $p < 0.001$ ) in the volume fraction of nutritive phagocytes from initial values (37.9% in males and 10.3% in females) to those in urchins at the end of the study (80.3% in males and 73.8% in females) (Fig. 2). The volume fractions of nutritive phagocytes in urchins maintained under ambient photoperiod did not change during this time ( $p = 0.376$ ).

Lengths of nutritive phagocytes increased significantly ( $p < 0.001$ ) in males and females from both

invariant and ambient photoperiod treatments (Fig. 3). Under invariant photoperiod there was an increase from  $86.3 \mu\text{m} \pm 7.3$  to  $261.4 \mu\text{m} \pm 8.64$  in males and from  $142.8 \mu\text{m} \pm 8.6$  to  $294.4 \mu\text{m} \pm 12.3$  in females. Nutritive phagocytes in urchins maintained under ambient photoperiod increased in length to  $217.0 \mu\text{m} \pm 16.3$  in males and  $408.5 \mu\text{m} \pm 19.6$  in females. When expressed

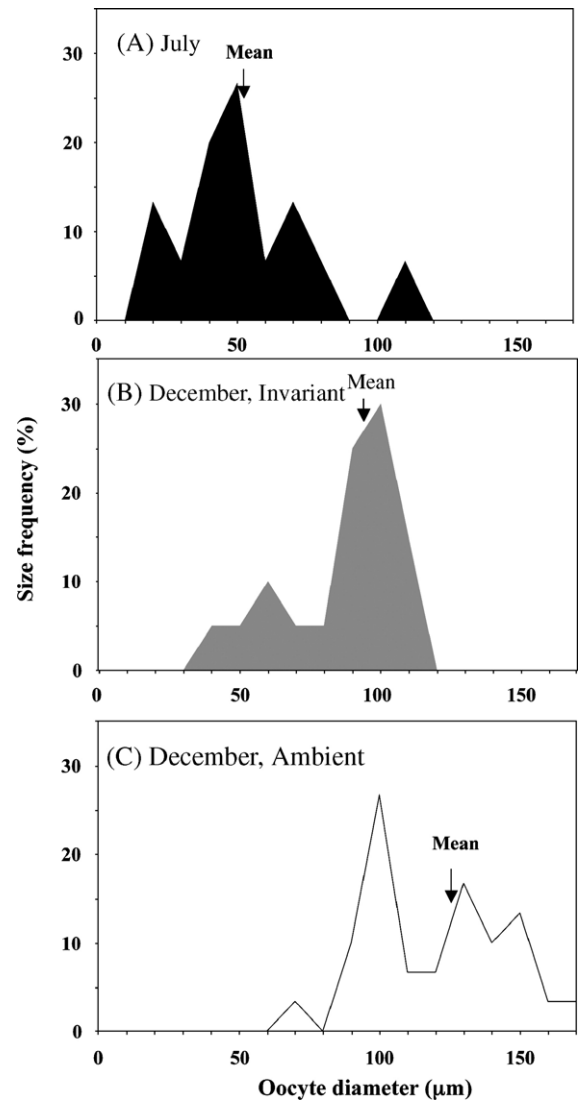


Fig. 4. Size frequency distribution (%) of the oocyte diameters for female *Strongylocentrotus droebachiensis* in (A) time zero urchins and urchins sacrificed in December (final measurement) after being maintained under (B) invariant (these were residual oocytes from last year's gametogenesis) and (C) ambient photoperiod (these included residual oocytes from last year's gametogenesis and new oocytes from this year's gametogenesis). (Arrows = mean oocyte diameter for each treatment; 30 oocytes measured/individual;  $n = 4$  individuals/treatment).

as the nutrient portion of the nutritive phagocytes (excluding the surrounding gametogenic cells in both sexes), NPs increased in length in males and females under invariant and ambient photoperiod. However, the nutrient portion of NPs in individuals under invariant photoperiod was significantly ( $p=0.008$ ) higher than that of individuals under ambient photoperiod.

There was a significant increase ( $p<0.001$ ) in the volume fraction of gonial cells from  $20.4\% \pm 5.5$  in males and 0% in females initially to  $37.8\% \pm 1.8$  in males and  $22.6\% \pm 3.6$  in females (Fig. 2) in individuals maintained for 5 months under ambient photoperiod.

Under invariant photoperiod, there was a significant decrease in the volume fraction of gonial cells to  $9.7\% \pm 2.1$  in males ( $p<0.001$ ) and no change in females ( $p=0.26$ ).

Volume fractions of luminal spermatozoa and of primary oocytes within NP incubation chambers (Fig. 2) also increased significantly ( $p<0.001$ ) in individuals maintained under ambient photoperiod from  $2.2\% \pm 1.3$  to  $28.0\% \pm 7.9$  in males and from  $18.6\% \pm 2.6$  to  $49.7\% \pm 3.6$  in females. There was no significant change in volume fractions of spermatozoa or residual oocytes in individuals maintained under invariant photoperiod ( $p=0.35$ ).

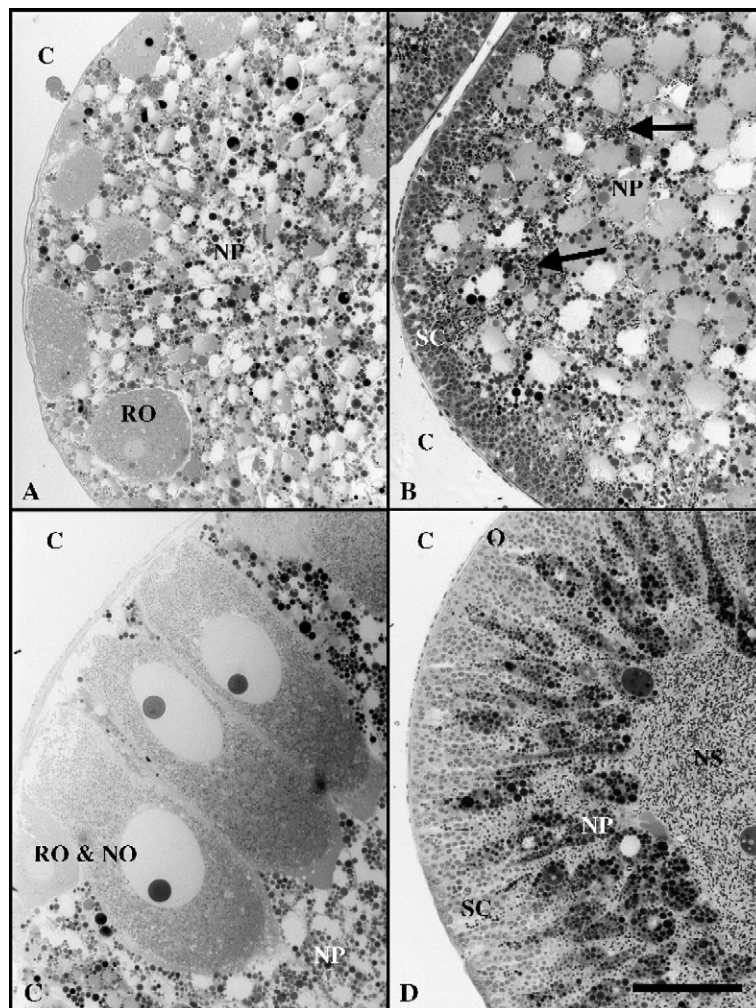


Fig. 5. Plastic sections of a representative *Strongylocentrotus droebachiensis* ovary (A) and testis (B) maintained for 5 months under invariant photoperiod showing pre-dominance of NPs and few gametes. Limited numbers of residual (RO) primary oocytes (no new ones) are present near the ovarian wall and limited numbers of new spermatozoa (arrows) are evident between the expanded NPs as are spermatogonial mitoses among the spermatogenic cells (SC). Plastic section of a representative *Strongylocentrotus droebachiensis* ovary (C) and testis (D) maintained for 5 months under ambient photoperiod showing growing residual (RO) and new (NO) primary oocytes in NP incubation chambers. The testicular lumen is filled with new spermatozoa and the NPs (dark granule containing cells) are reduced in size with only a slender strand of cytoplasm connecting them to the testicular wall; circle points out a spermatogonial mitosis (SC); scale bar=50  $\mu$ m; C, coelom.



Table 2

Taste testing results as determined by Atsushi Tamaki (ISF Trading Co., Hobson's Warf, Portland, ME)

	Ambient photoperiod	Invariant photoperiod
Taste	Sweet	Bitter
Color	Good orange yellow color	Males marketable, females brown color
Texture	Firm, marketable consistency	Too soft, consistency not marketable

When compared to oocytes in urchins at time zero, size frequencies (%) of oocyte diameters increased significantly in urchins maintained under both invariant and ambient photoperiod (Fig. 4). Under invariant photoperiod the mean diameter of residual oocytes from last years gametogenesis increased to  $93.5 \mu\text{m} \pm 3.7$  (from  $56.2 \mu\text{m} \pm 2.2$  initially). Under ambient photoperiod, mean oocyte diameters increased to  $126.0 \mu\text{m} \pm 7.3$ .

### 3.3. Histological and sensory analysis

Histological examination of plastic sections of gonads from both treatments clearly demonstrates differences in the cellular composition of the germinal epithelium. Ovaries (Fig. 5A) and testes (Fig. 5B) maintained under invariant photoperiod contained large nutrient filled NPs and a limited number of gametes in either sex. Some new spermatozoa were produced and limited numbers of residual primary oocytes (no obvious new ones) were present in NP incubation chambers. A layer of spermatogenic cells included mainly spermatogonia and a few were mitotic. *S. droebachiensis* ovaries (Fig. 5C) and testes (Fig. 5D) maintained under ambient photoperiod have new spermatozoa filling their lumen and a peripheral layer of spermatogenic cells with many mitotic spermatogonia. Growing residual and new primary oocytes were present within NP incubation chambers. NPs in both sexes were reduced in size as their nutrients are utilized in gametogenesis.

Sensory analysis of gonads from individuals maintained under invariant photoperiod in the land-based aquaculture system and fed a commercially available formulated urchin feed did not meet the expectations of our taste tester associated with the seafood industry (Mr. Atchan Tamaki, ISF Trading, Portland, ME). Their flavor was described as bitter and their texture was not sufficiently firm (Table 2). Gonads from urchins maintained at an open ocean lease site under ambient photoperiod treatment and fed the same formulated feed also had access to encrusting organisms and drift algae (as evident by their gut contents). Gonads from these urchins had a sweet taste and good texture (Table 2).

## 4. Discussion

As Dumont et al. (2006) point out, initiation of gametogenesis in some species of sea urchins is governed by short day-lengths (e.g., *S. purpuratus* and *S. droebachiensis*, *Eucidaris tribuloides*) while others require long day-lengths (e.g., *Psammechinus miliaris* (Kelley, 2001); *Paracentrotus lividus*). Here we evaluate the effects of invariant summer “vs” progressing ambient (fall/winter) photoperiod regimes on the gonads of *S. droebachiensis* fed a commercially available formulated feed. Results indicate that maintenance under invariant photoperiod yields gonads in both sexes of *S. droebachiensis* that do not initiate annual gametogenesis (although some new spermatozoa are produced, Walker et al., 2005). Gonads of both sexes do attain large size as a result of the growth of their nutritive phagocytes (and in ovaries, growth of limited numbers of residual primary oocytes remaining from last year's gametogenesis, Walker et al., 2005). Finally, gonad taste was unsatisfactory in urchins under invariant photoperiod. Gametogenesis was initiated and progressed normally (Walker et al., 2005) in the gonads of urchins maintained under ambient photoperiod and taste was satisfactory. In the latter, taste was favorably influenced by the availability of encrusting organisms and drift algae (concluded from the presence of kelp and mussel fragments in the gut of dissected individuals from the ambient photoperiod treatment only).

Differences in volume fractions of cellular components of the germinal epithelium were evident in gonads maintained under the photoperiod treatments employed in this study. Continuous July photoperiod leads to an increase in the volume fraction of NPs, a decrease or status quo in production of gonial/gametogenic cells, a smaller mean oocyte diameter and no new oocytes compared to individuals maintained under ambient photoperiod. During the month of July, *S. droebachiensis* are in the early Pre-Gametogenesis and NP Renewal Stage and contain NPs that are accumulating nutrients (Walker et al., 2005, 2006). In *S. droebachiensis* collected from wild populations towards the end of this stage (August/September), gonads with these characteristics have previously been shown by sensory panels to yield the most satisfactory sensory scores (Lee and Haard, 1982) and yet such gonads have not attained the largest size for the year. Urchins of varying commercial value occur in the wild from late September through early January during the Gametogenesis and NP Utilization Stage of gametogenesis. Later in this stage, the ratio of NPs to gametes shifts in favor of gametes and sensory scores are lowest for the year (Lee and Haard, 1982). In the current

study, we successfully used invariant photoperiod to generate large gonads, based on the pre-dominance of NPs in the germinal epithelium at a time of year when they would normally be diminished in size in wild populations.

A fortuitous result of maintaining urchins in ambient photoperiod in cages was their access to an additional food source resulting in their satisfactory sensory evaluation. This result suggests that the only commercially available sea urchin formulated feed requires modification to support continued development of this form of land-based aquaculture. However, an additional treatment of troughs with ambient photoperiod under the same fluorescent lights would eliminate the possible, but unlikely, effects of flow rate, urchin density, type of lighting, etc. on sensory evaluation of the gonads of *S. droebachiensis*.

Results of this study also emphasize that the most significant problem associated with edible sea urchin aquaculture is the production of large urchin gonads simultaneously characterized by consistently high quality sensory evaluations for taste, texture, color and firmness (Walker et al., 2006). Since the cellular details of gametogenesis are poorly known for most sea urchins and have only recently been elaborated for *S. droebachiensis* (Walker et al., 2005), it has not previously been possible to develop formulated feeds that take into account the actual nutrient requirements of sea urchins at particular stages of gametogenesis. Instead existing formulated feeds emphasize maximizing color and size of the commercial product. Satisfactory gonad color can be obtained using  $\beta$ -carotene (Pearce et al., 2003; Robinson et al., 2002, 2004), a pre-cursor to echinenone that is responsible for the color of sea urchin gonads (Griffiths and Perrott, 1976). Laboratory raised sea urchins demonstrate that formulated feeds are superior to a kelp diet in optimizing gonad size based mainly on increased levels of protein (Fernandez and Pergent, 1998; Pantazis et al., 2000; Pearce et al., 2002a,b,c; Carcamo, 2004; James et al., 2004; Schlosser et al., 2005). However, indiscriminately increasing protein levels can yield bitter tasting gonads (Hirano et al., 1978; de Jong-Westman et al., 1995; Hoshikawa et al., 1998; Hammer et al., 2006; Pearce et al., 2002b and this study). Also, none of the available formulated sea urchin feeds (including the one we used in this study) has been subjected to rigorous testing of taste and other sensory parameters by a professional panel of taste testers. Obviously large gonads that do not meet the exacting standards of Japanese and other consumers are of little immediate value and could even be counterproductive to the long-term commercial development of a viable sea urchin aquaculture industry.

In the Pre-Gametogenesis and NP Utilization Stage in the wild, the pre-dominant amino acids in the gonads of both sexes are glycine, arginine, lysine, leucine, tyrosine, valine and isoleucine (Komata et al., 1962; Lee and Haard, 1982). As gametogenesis begins, levels of these and other amino acids drop sharply and sensory evaluations are less positive. Formulated feeds need to be designed that limit protein concentrations to maintenance levels required for optimal growth of NPs and that contain the amino acids known to be prevalent in NPs that are storing nutrients. If our approach of using invariant photoperiod to generate large gonads, based on the pre-dominance of NPs in the germinal epithelium could be coupled with a formulated feed that yields large gonad size (based on the pre-dominance of NPs) and simultaneously results in a satisfactory sensory evaluation, such technology could be of great value in the development of land-based commercial sea urchin aquaculture.

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