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Photoperiodic Regulation of Gametogenesis in Sea Stars, with Evidence for an Annual Calendar Independent of Fixed Daylength[1,2]

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SYNOPSIS. Gametogenesis and gonadal growth in the west coast sea star *Pisaster ochraceus* normally begins in the fall and leads to large gonads full of gametes in the spring, when spawning occurs. The timing of gametogenesis can be shifted simply by maintaining the animals on a seasonally changing photoperiodic regime out of phase with ambient. When they are kept on a spring-summer photoperiodic regime during the fall and winter, gametogenesis proceeds 6 mo ahead of schedule in the following spring and summer. Gametogenesis can be shifted out of phase even when the eyespots are removed. Short daylengths that normally occur during the fall and winter are not required for gametogenesis to proceed, nor are even the long daylengths of spring and summer that precede the initiation of gametogenesis in the fall. The temporal program is insensitive to fixed daylengths (LD 15:9, 13:11, 12:12, 9:15) and appears to involve an endogenous calendar.

Shifting the photoperiodic regime 6 mo out of phase also leads to a shift of the gametogenic temporal program in the sea stars *Leptasterias* sp. (a brooder) and *Asterias vulgaris* (from the New England coast), but not in the sea star *Patiria miniata*. Gametogenic timing also can be switched in the sea urchin *Strongylocentrotus purpuratus* but the mechanism of the photoperiodic response is fundamentally different; gametogenesis requires short daylengths; continues indefinitely under a repeated short day, fall-winter photoperiod regime, and apparently does not involve an endogenous calendar. As photoperiodic responses are investigated further in these and other marine invertebrates, the models developed primarily from studies on terrestrial organisms may need to be extensively modified or additional new models required.

INTRODUCTION

The seasonal production of gametes by sea urchins, sea stars, and other marine animals has been a familiar fact of life for well over a century, particularly to cell and developmental biologists who have depended on the gametes of these animals for research material. Until recently, however, little attention has been paid to the environmental and physiological mechanisms that control this seasonality, or to the ecological and evolutionary mechanisms that maintain it. Orton's (1920) classic paper pointed to seasonal changes of sea temperature as the major environmental factor determining the timing of reproductive activity of marine animals. Sufficient correlative evidence accumulated over the following decades for Thorson (1946) to propose that Orton's suggestion take on the status of a general rule ("Orton's Rule"); subsequent experimental work showed that changes in sea temperature can modify reproductive timing in many marine species, especially in the North Atlantic (reviewed by Giese and Pearse, 1974). Nevertheless, seasonally changing sea temperatures are not likely to be very influential in controlling the timing of reproduction in species that live in areas where sea temperature undergoes little seasonal change (*e.g.*, the west coast of much of North America). Moreover, even in areas with substantial seasonal change of sea temperature, the sequence of events involved in gamete production often seems too precisely synchronized from place to place and year to year to be determined solely by changing sea temperatures.

The importance of seasonally changing photoperiods in synchronizing reproductive activities of terrestrial plants and ani-

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[2] We dedicate this paper to Professor Arthur C. Giese on his 80th birthday, 19 December 1984.

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mals has been recognized for nearly 50 years, and a large and cumbersome literature has accumulated on various ramifications of the system. However, despite Giese's (1959) suggestion that photoperiodism may be important in the sea as well, and work by Richard (1971) signalling such importance, its role has only recently been recognized and more thoroughly examined in both marine plants and animals (see papers in this symposium).

Sea urchins and sea stars recently have joined the ranks of animals known to have photoperiodic responses. When reared in a photoperiodic regime of seasonally changing daylengths that is 6 mo out of phase with ambient (*i.e.*, the longest day of the year is 21 December), individuals of the west coast sea urchin *Strongylocentrotus purpuratus* both grow and produce gametes 6 mo out of phase with those in the field and those reared in a seasonally changing photoperiod regime in phase with that in the field (Pearse *et al.*, 1986). Gametogenic activity is maintained, apparently continuously, when animals are kept on repeated short-day fall-winter photoperiod regimes, but suppressed when they are kept on repeated long-day spring-summer photoperiod regimes. The animals thus appear to be typical "short-day" organisms with respect to gametogenesis, and there is no evidence that they possess an endogenous rhythm or calendar controlling growth or gametogenesis.

Photoperiodic control of growth of storage organs (pyloric ceca) and gonads also has been demonstrated in several species of sea stars, including *Pisaster ochraceus* by Pearse and Eernisse (1982) and *Asterias vulgaris* by Pearse and Walker (1986), both species with planktotrophic larvae, and *Leptasterias* sp. by Pearse and Beauchamp (1986), a brooding species without larvae. Both *P. ochraceus* and *Leptasterias* sp. occur on the west coast of North America where temperature fluctuations are moderate, but *A. vulgaris* occurs on the northeast coast of North America where seasonal temperatures range over nearly 20°C. These observations suggest that photoperiodic control of gametogenesis is widespread in shallow-water sea stars. However, we found no evidence of photoperiodic control of gametogenesis in another common west coast sea star, *Patiria miniata* (K. K. Davis and J. S. Pearse, unpublished observation).

Continuing work in our laboratory has revealed that the photoperiodic response in *Pisaster ochraceus* is unlike that in *Strongylocentrotus purpuratus.* Individuals of *P. ochraceus* appear to be unresponsive to the short days of fall and winter, but rather can be caused to produce gametes out of phase by exposure to the long days of spring and summer. Although they thus seem to be "long-day" animals, they are unresponsive to fixed daylengths, either short, neutral, or long, nor are long daylengths of spring and summer necessary for gametogenesis to proceed in the fall. There is evidence in these animals for an endogenous calendar, which is perhaps set very early in life and which underlies the temporal program of gametogenesis. This symposium paper summarizes some of our work on *P. ochraceus* that is aimed at characterizing the nature of the photoperiodic response in these animals, and places it in perspective with current ideas about photoperiodic phenomena.

MATERIALS AND METHODS

Our basic experimental protocol is given in Pearse and Eernisse (1982) and Pearse *et al.* (1986). Individuals of the sea star *Pisaster ochraceus,* common intertidally on the Pacific coast of North America, were collected from an intertidal mussel bed in Santa Cruz, California. These animals were maintained in tanks with running seawater under identical conditions of food (unlimited supply of mussels upon which all fed nearly continuously), of sea temperature (ambient; ranging from 10–16°C in winter and spring to 12–18°C in summer and fall, see Pearse and Beauchamp, 1986), and of density but under contrasting photoperiodic regimes. In one light-tight room an astronomic time switch (R. W. Cramer & Co., Type SY Model SOL) turned the fluorescent lights (G.E. F40D Daylight) on and off in phase with local sunrise and sunset; the animals in this room were under an "ambient" or "in-phase" photoperiodic regime. In the adjacent room the astro-

nomic time switch was set to turn the fluorescent lights on and off 6 mo out of phase with local sunrise and sunset so that the longest day of the year was on 21 December and the shortest day was 21 June; animals in this room were under an "out-of-phase" photoperiodic regime. Behavior, feeding, growth, and gametogenic activity were monitored in animals maintained in the two rooms. Subsamples of 2 to 7 animals were taken on a quarterly to semiannual schedule, gonadal indexes determined (gonadal index = gonadal wet weight × 100/total animal wet weight), and the gonads prepared and analyzed histologically. As found in earlier studies (*e.g.*, Giese, 1959), there was no statistical difference between the gonadal indexes of males and females taken on any given date; the values therefore were combined for plotting and analyses.

In the first experiment, lasting from December 1978 to August 1980, animals were simply maintained in separate rooms under one or the other of the contrasting photoperiodic regimes; this experiment demonstrated photoperiodic control of gametogenesis in these animals as reported by Pearse and Eernisse (1982). In the second experiment, using 60 animals and lasting from March 1980 to December 1981, individuals were transferred from one of the light-tight photoperiod rooms to the other at 6 mo intervals; some experienced repeated spring-summer regimes ("long day") while others experienced repeated fall-winter regimes ("short day"). In the third set of experiments, lasting from December 1981 to August 1983, 192 individuals were maintained 16 each in 12 plastic laundry sinks (50 × 50 × 35 cm) covered with light-tight wooden boxes, each equipped with an overhead 24-inch fluorescent light (G.E. F24D Daylight). For some of the boxes, the astronomic timers were set to produce seasonally changing photoperiods so that there were in-phase (ambient), 3-mo out-of-phase, and 6-mo out-of-phase regimes. For others, 24-hour timers (Intermatic, Models D-111 and D-811) were set to produce fixed daily hours of light (L) and dark (D)—LD 15:9, 13:11, 12:12, and 9:15. In addition, to examine the possibility that an "hourglass" model of photoperiodic control is involved (see Saunders, 1982), long days were interrupted by 1 and 5 hours of darkness every day (*i.e.*, LDLD 8:1:7:8 and 8:5:7:4). Finally, animals in one box were initially exposed to a fixed photoperiod of LD 15:9 for one month, then to LD 9:15 for the remainder of the experiment.

In the experiments described above, the animals were killed upon dissection, so the length of an experiment was determined in part by the initial number of animals maintained (12 or more per treatment), the frequency of subsampling, and the number of animals in the subsamples. In order to include both males and females in the subsamples, animals were sexed by examining gonadal tissue withdrawn with a hypodermic needle and syringe. In several additional experiments small numbers of juveniles were reared and maintained for four to five years (the "entrainment experiments" presented at the end of the Results section); these individuals were biopsied semiannually, but not killed. Initially, a single gonad was removed from each animal through a small slit cut in the base of a ray, weighed to estimate the gonadal index (assuming all gonads had similar weights), and then fixed for histological analysis. However, often the wound did not heal, and the slit remained as an opening into the coelom. Such animals appeared collapsed and stressed, and they died within weeks to months. Later biopsies were done by cutting off one whole ray with a razor blade and removing the gonads from that ray. After ray-removal, the animals healed well, and the regenerating ray bud reached a centimeter or more in length within about 6 mo. The effect of regeneration on gametogenesis is unknown; it probably does not change gametogenic timing, but may depress gonadal growth (Harrold and Pearse, 1980).

RESULTS

Switching experiments: Repeated "winters" and "summers"

Animals collected in March, when they contained large gonads full of ripe gametes, all spawned in the laboratory in June

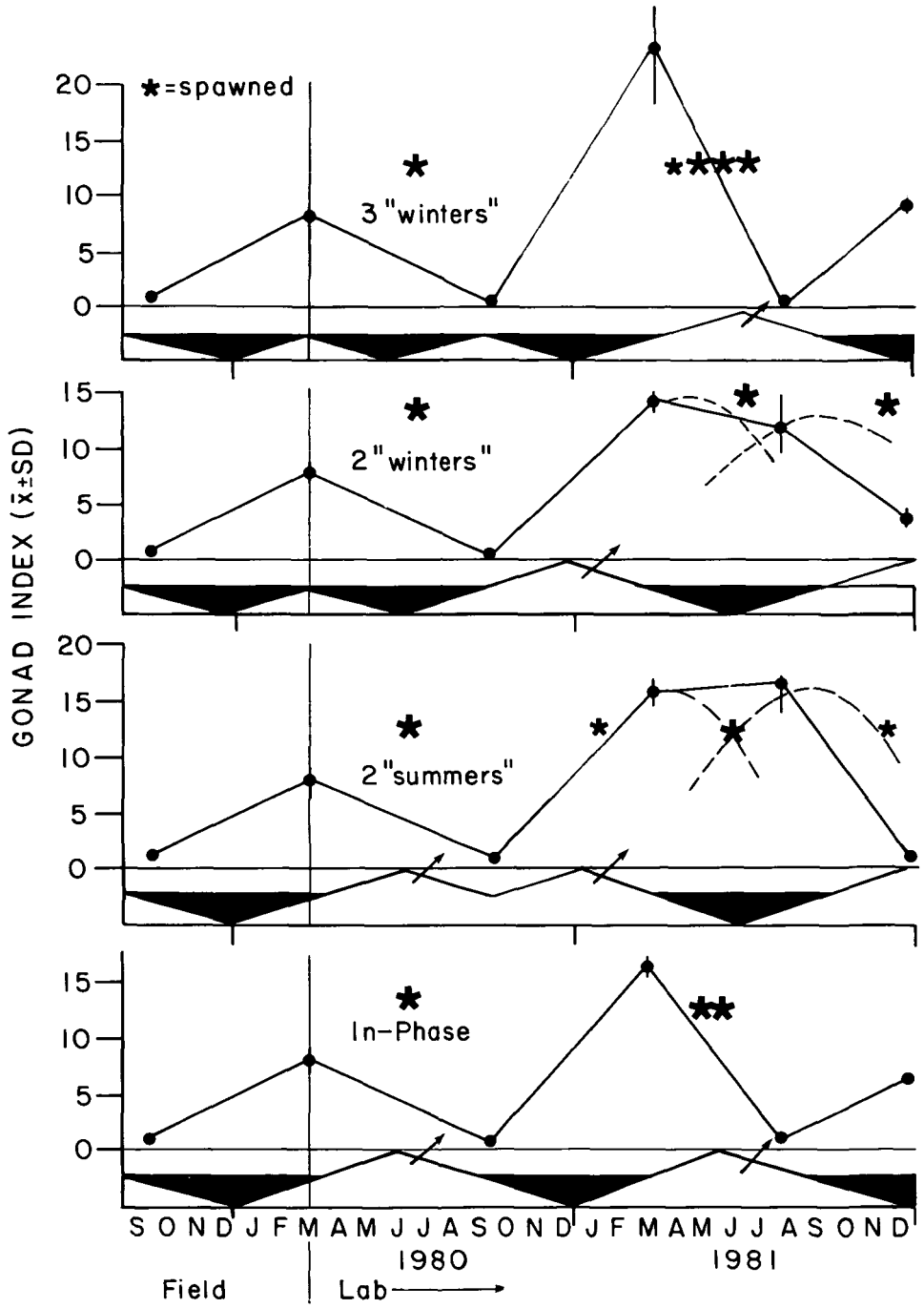

FIG. 1. Changes in the gonadal indexes (males and females combined) of *Pisaster ochraceus* maintained on different regimes of seasonally changing photoperiods. Black areas represent periods when daylengths were less than 12 hr; photoperiods ranged from 9 hr at winter solstice to 15 hr at summer solstice as indicated at the bottom of each panel. Sixty animals were brought into the laboratory in March 1980 and divided equally between photoperiod regimes that were in phase (lower two panels) and out of phase (upper two panels); half of each group was switched to the alternate photoperiodic regime in September 1980. Sample sizes for each dissection date were: September 1979 and March 1980, 10 animals (field samples); September 1980, 5 animals

regardless of photoperiodic regimes, and all had very small gonads in September (Fig. 1). Moreover, *all* underwent a gametogenic cycle between September and March, regardless of photoperiod regime, including those transferred from an in-phase to an out-of-phase regime ("2 summers"), those maintained out-of-phase ("2 winters"), and those transferred from an out-of-phase to an in-phase regime ("3 winters"). During the following year, those returned to in-phase conditions after being maintained for 6 mo under out-of-phase conditions ("3 winters") followed the in-phase temporal program. In contrast, those maintained under the out-of-phase regime, beginning either in March ("2 winters") or in September after being held on the in-phase regime for 6 mo ("2 summers"), underwent a second gametogenic cycle in the spring and had large gonads filled with full-grown oocytes or sperms in August 1981. These animals were seen spawning in December, 6 mo out of phase with the in-phase animals, and had small undeveloped gonads upon the final December dissection.

These experiments show that the gametogenic cycle (both in ovaries and testes) in these animals can proceed 1) whether or not it is preceded by the long days of spring and summer, and 2) whether or not it is accompanied by the short days of fall and winter. Moreover, as found earlier (Pearse and Eernisse, 1982), when the animals were maintained under long days of spring and summer during the fall and winter they initiated a gametogenic cycle 6 mo early.

Phase-shifting experiments: 3-mo and 6-mo out-of-phase regimes

Although Pearse and Eernisse (1982) had demonstrated that the gametogenic program in *Pisaster ochraceus* could be shifted 6 mo out of phase by placing the animals under a photoperiod regime 6 mo out of phase, we needed also to determine the flexibility of the program. Could it be reset to any phase? After animals had been maintained for 20 mo on a photoperiod regime 3 mo out of phase with ambient, their gonadal size and oogenic condition (estimated by analysis of oocyte sizes) were intermediate between those of animals maintained on in-phase and 6-mo out-of-phase regimes (Fig. 2). The shift, and the intermediate status of the 3-mo out-of-phase animals, was evident within a year, in December, when those 6 mo out of phase were seen spawning; the 3-mo out-of-phase animals were seen spawning in February and April, and those under the in-phase regime spawned in May, June, and July.

It should be noted that for unknown reasons gonadal growth was suppressed during the first spring in animals maintained under both 3-mo and 6-mo out-of-phase regimes, although all the animals spawned between May and July (Fig. 2). A similar, but more severe suppression (the animals did not spawn the first year) was seen in animals maintained under an interrupted fixed photoperiod (LDLD 8:1:7:8).

The role of the terminal eyespots

Many sea stars, including *Pisaster ochraceus,* have an ocellus at the tip of each ray. Variation in light received by these ocelli modifies sea star behavior (Yoshida and Ohtsuki, 1966). Moreover, transmission electronmicroscopic studies have demonstrated a nightly rhythm of membrane regeneration in the ocellar cells (Brandenburger and Eakin, 1980). Such a circadian rhythm could provide a mechanism for measuring seasonally changing daylength (Pittendrigh, 1981*a*).

We tested the possibility that the eyespots serve as the receptors for the photoperiodic response by surgically removing the eyes and a small portion of tissue around them (several mm^2, including the terminal

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per treatment (in phase and out of phase); March, August, and December 1981, 4 animals per treatment. (The remaining two provided the authors with an excellent dinner.) Arrows indicate when long spring-summer daylengths apparently influence the initiation of gametogenic cycles; dotted lines suggest course of overlapping gametogenic cycles.

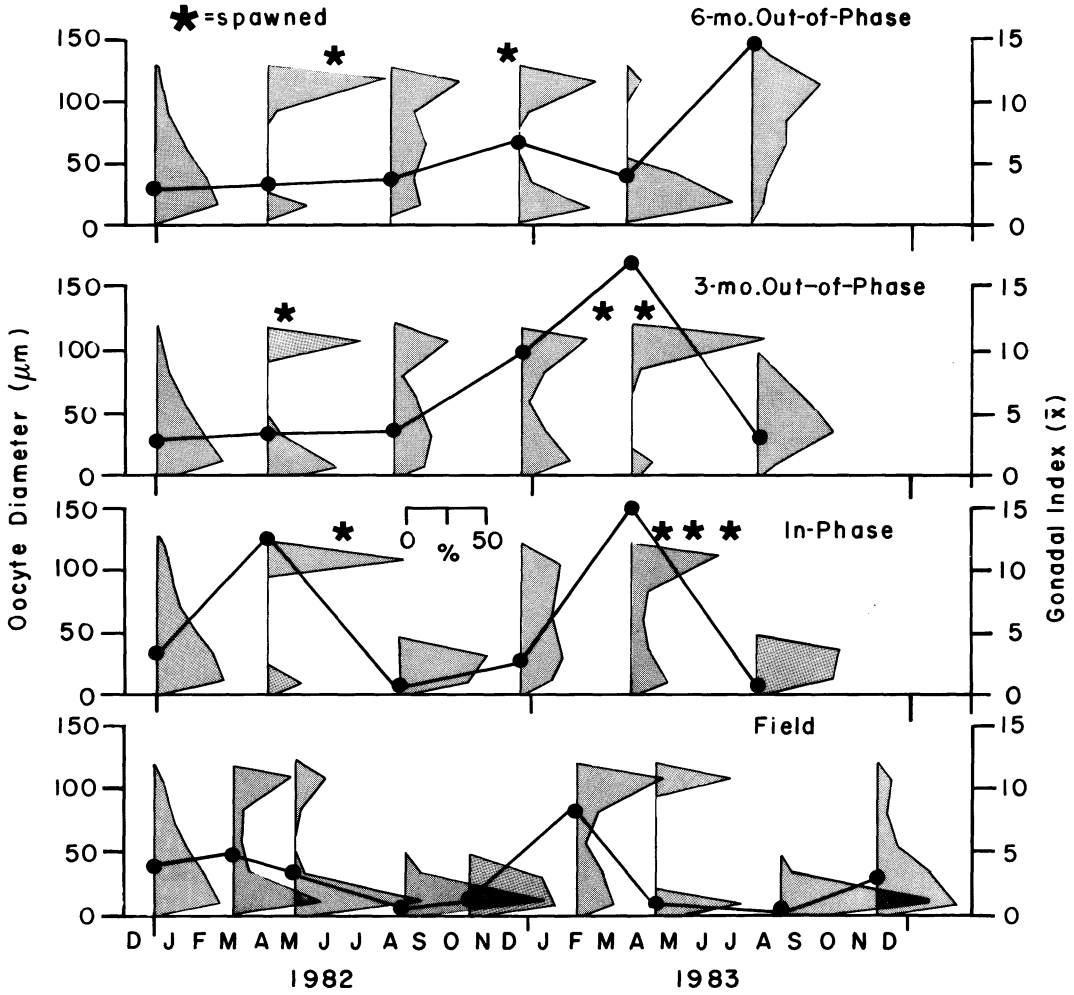

FIG. 2. Changes in gonadal indexes (solid lines) and size distributions of oocytes (shaded polygons) of *Pisaster ochraceus* maintained under seasonally changing photoperiods with different phases. Animals in 3-mo out-of-phase treatment were initially shifted ahead from mid-December photoperiod to that equivalent of mid-March; those in 6-mo out-of-phase treatment were shifted to equivalent of mid-June. Initial sample size 10 animals, thereafter field samples ranged from 5 to 10 animals, and laboratory samples ranged from 2 to 6 animals; final laboratory sample sizes were 5 to 6 animals per treatment. Field animals presumably spawned between February and June each year.

tube feet). Treated animals then were maintained under both in-phase and 6-mo out-of-phase photoperiodic regimes. Regeneration of the eyespots is rapid (see Penn and Alexander, 1980); we found small regenerated ocelli within 3 to 4 weeks after removal. Consequently, we removed the eyespots every 3 weeks. The behavior of the surgically treated animals was affected; they rarely moved around the tanks and their feeding rates were less than half those of untreated animals.

Despite the obvious adverse effects of eyespot removal, the gametogenic cycle was virtually identical between treated and untreated animals maintained under the in-phase regime (Fig. 3). However, those with the eyespots removed were never seen to spawn, and the gametes remaining in the gonads of the August subsample of treated animals were disintegrating relicts. Perhaps the eyespots have a role in spawning (but see below).

The gametogenic cycle also was very

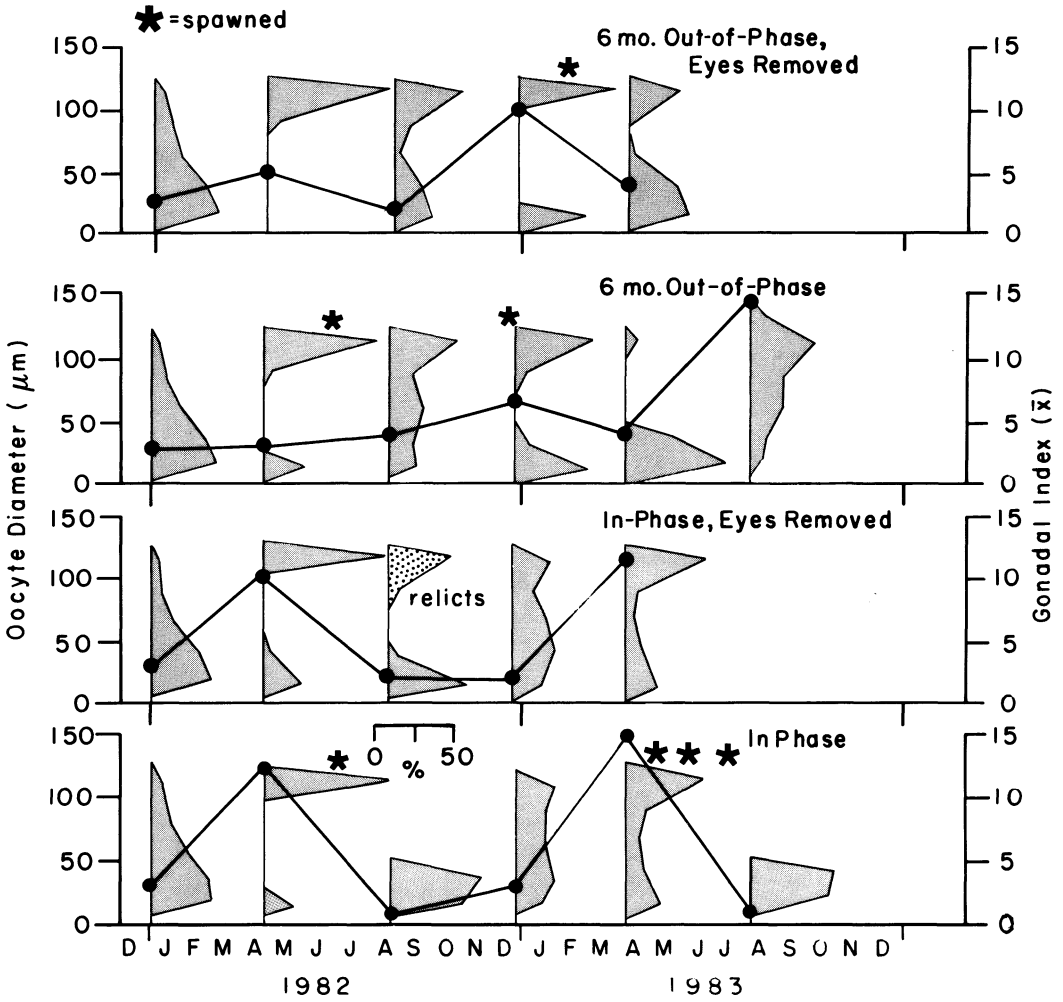

FIG. 3. Changes in gonadal indexes (solid lines) and size distributions of oocytes (shaded polygons) of *Pisaster ochraceus* maintained under seasonally changing photoperiods that were in phase and 6 mo out of phase. The eyes (ocelli) at the tips of the rays were removed surgically every 3 to 4 weeks from one set of animals maintained under each photoperiod regime. "Relicts" refers to oocytes that were disintegrating, as seen in histological preparations. Sample sizes for the April 1983 dissections were 7 animals each for the eye-removal treatments and 4 animals each for the eye-intact treatments.

similar between treated and untreated animals maintained under the 6-mo out-of-phase regime; in both sets of animals it had shifted out of phase (Fig. 3). This result suggests that the eyespots are not the photoreceptors responsible for mediating the photoperiodic response of gametogenesis. Moreover, although the treated, out-of-phase animals did not spawn the first year, a few were seen spawning in January of their second year, showing that spawning can occur even in the absence of the ocelli.

Fixed daylengths

The gametogenic cycles were virtually indistinguishable among animals maintained under fixed daylengths (Fig. 4), including long days (LD 15:9, and 13:11 not shown), neutral days (LD 12:12), and short days (LD 9:15); these cycles were essentially the same as those in animals maintained under the in-phase photoperiodic regime. Only two differences were noted. 1) Animals under long-day and neu-

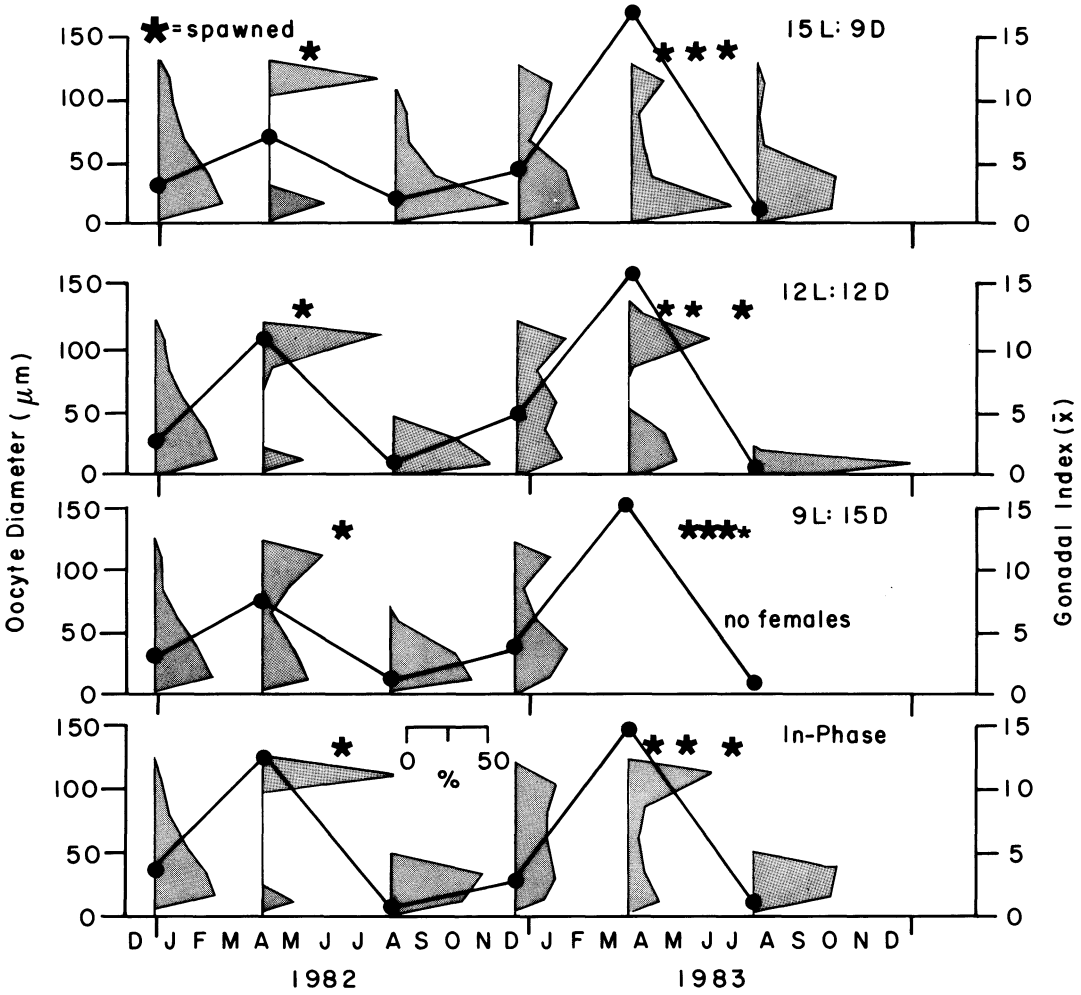

FIG. 4. Changes in gonadal indexes (solid lines) and size distributions of oocytes (shaded polygons) of *Pisaster ochraceus* maintained under fixed daylengths in contrast with those in animals maintained under seasonally changing in-phase photoperiods. Final sample sizes for the August 1983 dissections were 6 animals for the LD 15:9, 9:15, and in-phase treatments, and 3 animals for the LD 12:12 treatment.

tral regimes spawned one to two months before short-day animals, again indicating a possible role of light in spawning. 2) Vitellogenic oocytes, more than 50 μm in diameter, were found in August of both years only in the ovaries of long-day animals (both LD 15:9 and 13:11). This observation suggests that unseasonal gametogenic activity is stimulated by long daylengths, but is subsequently suppressed by other factors.

The gametogenic cycles of animals maintained on fixed but interrupted long-day regimes also were similar to those of in-phase animals (Fig. 5). However, as with the animals on fixed long-day regimes, animals on fixed "interrupted" regimes had vitellogenic oocytes above 50 μm in diameter in August of both years. Moreover, in the animals maintained on the LDLD 8:1:7:8 regime, the gametogenic cycle already in progress at the start of the experiment was severely suppressed, spawning was not seen the first year, and spawning was several months later the second year than in other long-day, neutral-

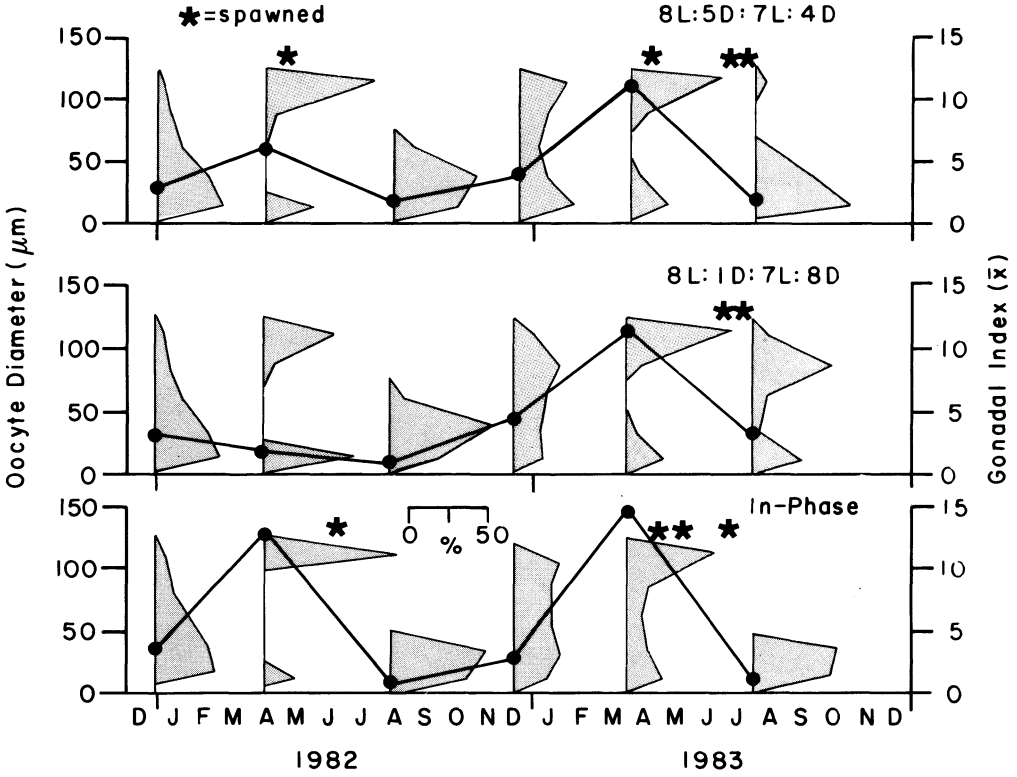

FIG. 5. Changes in gonadal indexes (solid lines) and size distributions of oocytes (shaded polygons) of *Pisaster ochraceus* maintained under interrupted, fixed, long daylengths in contrast with those in animals maintained under seasonally changing in-phase photoperiods. Final sample sizes for the August 1983 dissections ranged from 5 to 7 animals.

day, and in-phase animals. It is not clear how to interpret these findings.

Never was a complete out-of-phase gametogenic cycle produced by placing in-phase animals into any of the continuous fixed-daylength regimes. However, one such cycle appeared to result when animals were placed under long days (LD 15:9) for one month in December–January, then transferred to and maintained under a continuous short-day regime (LD 9:15) (Fig. 6). In this case large vitellogenic and full-grown oocytes were found in the August sample of the first year and spawning occurred in December, as in the animals held 6 mo out of phase. However, the animals also had large gonads full of gametes in April of the second year and their gonads were small and their ovaries contained mainly very small oocytes in August of the second year, as in the animals held in phase. The brief period of unseasonal long days seems to have stimulated a single, extra, out-of-phase gametogenic cycle.

Entrainment experiments

The above experiments, that using neutral daylengths, in particular, indicate that the gametogenic cycle in *Pisaster ochraceus* is at least partly independent of photoperiod. There are at least two possible explanations. In the absence of photoperiod cues, the animals could 1) follow changes in some other environmental variable, such as in sea temperature or food quality, or 2) follow changes in an endogenous calendar, analogous to the self-sustaining oscillators in circadian clocks (Pittendrigh, 1981*b*). If an endogenous calendar is present, it might be possible to initiate or set it out of phase. Evidence for such a calendar would be the finding that animals set out of phase would

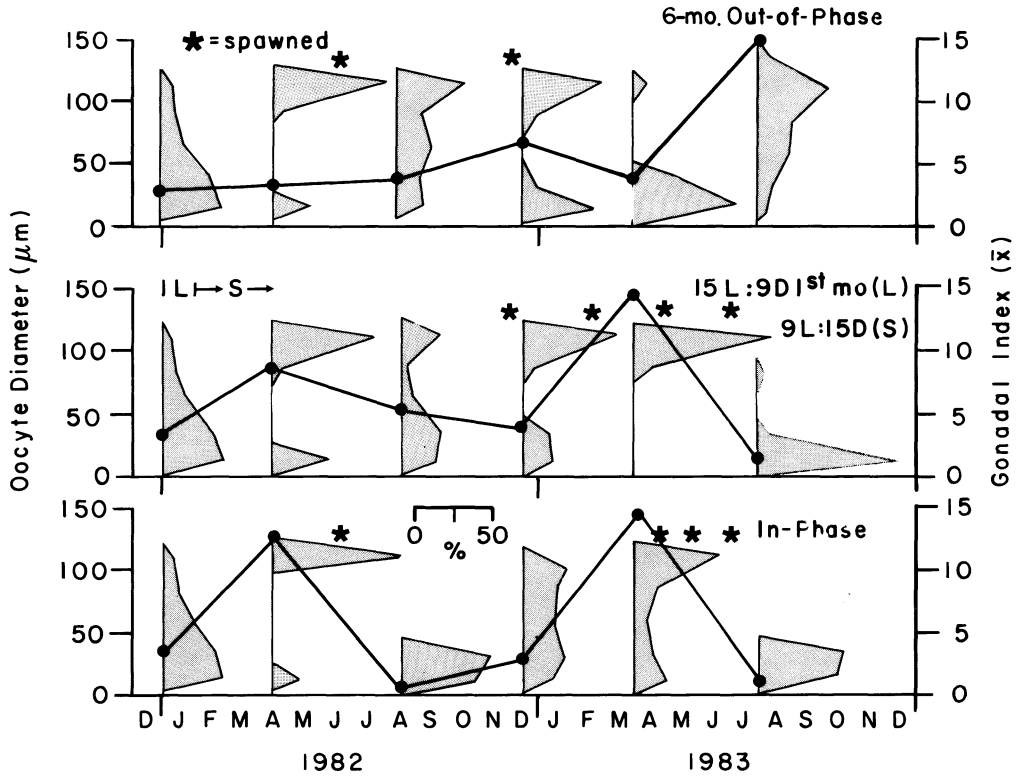

FIG. 6. Changes in gonadal indexes (solid lines) and size distributions of oocytes (shaded polygons) of *Pisaster ochraceus* maintained for one mo under fixed long daylengths (LD 15:9), then for the remainder of the experiment under fixed short daylengths (LD 9:15), in contrast with those in animals maintained under seasonally changing photoperiods that were in phase and 6 mo out of phase with ambient photoperiods. Final sample sizes for the August 1983 dissection ranged from 5 to 6 animals.

remain out of phase in the absence of photoperiodic cues (*i.e.*, when maintained at LD 12:12), even when kept under identical conditions of sea temperature and food supply as in-phase animals.

We made two experimental attempts to entrain animals out of phase and then test their ability to maintain an out-of-phase cycle under ambient sea temperatures and constant food supplies. Juveniles between 5 and 20 g wet weight were used in both of these experiments in an attempt to avoid already entrained cycles. Gonads do not begin growth in animals less than 50–75 g, and gametes have not been found in animals less than 100 g (J. S. Pearse, unpublished data).

In one experiment 12 juveniles were collected in March 1981 and divided equally between the in-phase and out-of-phase rooms. Because of their small sizes they were maintained in plastic salad collanders and fed mussels less than 1 cm in length. After one year, when they had grown to a mean wet weight of 124 g, they were transferred to plastic dish pans, and the two pans were placed side-by-side in the out-of-phase room. Subsequently, every September and every March, both pans of animals were transferred to the alternate rooms so that the animals experienced repeated fall-winter photoperiod regimes, but not the long days of spring and summer that can reset the gametogenic cycle (Fig. 7, top).

The animals were first biopsied in March 1983, after they had been held for 2 years and had reached a mean wet weight of 480 g; the mean gonadal index (calculated by multiplying the wet weight of one gonad by 10) of the in-phase entrained animals

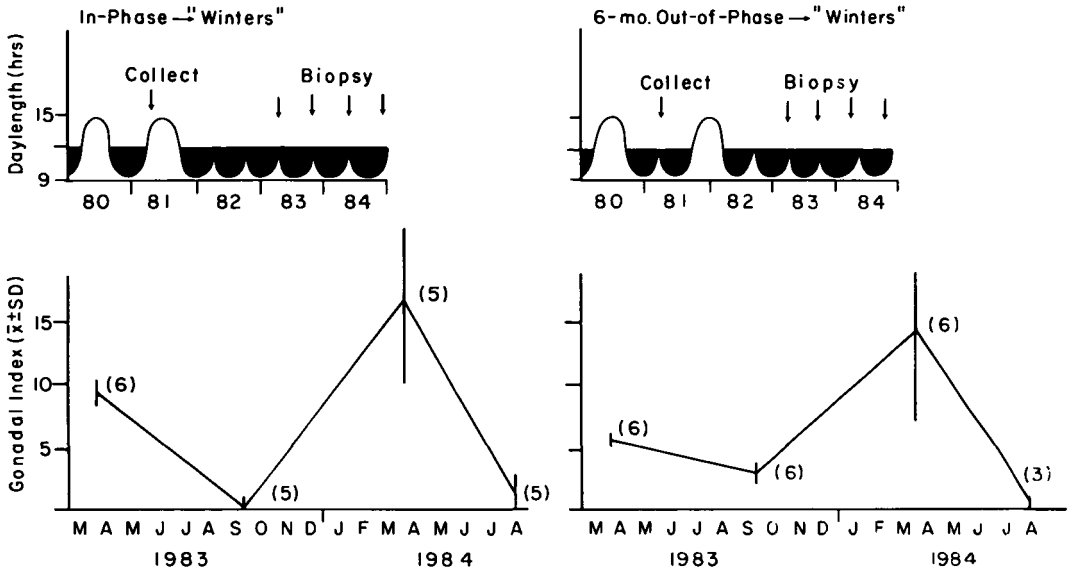

FIG. 7. Changes in gonadal indexes of *Pisaster ochraceus* reared for one year under seasonally changing photoperiods that were in phase or 6 mo out of phase with ambient photoperiods, then maintained under repeated fall-winter photoperiods with daylengths less than 12 hr. Numbers in parentheses give number of animals surviving and sampled by biopsy on each date; all surviving animals were biopsied on each successive sampling date. Note temporal scales in top and bottom figures do not correspond.

was significantly ($P < 0.05$) larger than that of the out-of-phase entrained animals (Fig. 7, bottom); however, that of the latter was larger than would be expected, and the gonads of both sets of animals contained numerous oocytes of various sizes or spermatocytes and sperms, and they were indistinguishable histologically. In September 1983, the gonadal index of the out-of-phase entrained animals was significantly larger than that of the in-phase entrained animals. Moreover, unlike the gonads of the in-phase entrained animals, those of the out-of-phase animals were full of full-grown oocytes or sperms. Subsequent biopsies in March and August 1984, however, showed no differences between the two sets of animals, and both sets followed the in-phase course of gametogenesis.

In the other entrainment experiment, 5 juveniles were collected in March 1980 and reared in the out-of-phase room for three years. Three other juveniles were reared in the in-phase room beginning in March 1981. All these animals were biopsied in March 1983 and then placed under a neutral photoperiod (LD 12:12), after which they were biopsied semiannually (Fig. 8). From March 1983 to March 1984, the two sets of animals remained 6 mo out of phase with each other; the in-phase entrained animals had large gonads full of gametes in March of 1983 and 1984, and very small gonads with very few gametes in September 1983; the out-of-phase entrained animals had large gonads full of gametes only in September 1983. However, by August 1984, the remaining animals, all of which were entrained to be out of phase, had gonads that were in every way like those of in-phase animals. Mortality of the biopsied animals was high between March and August 1984 when three of the six animals in the experiment died, perhaps as a result of the repeated biopsies. Whether the small size and undeveloped state of the gonads in these animals were due to their reverting to in-phase conditions or to stress, therefore, is unclear.

These entrainment experiments were intriguing yet inconclusive. In both experiments, the gametogenic cycle in animals entrained out of phase remained at least partly out of phase for the first year, indi-

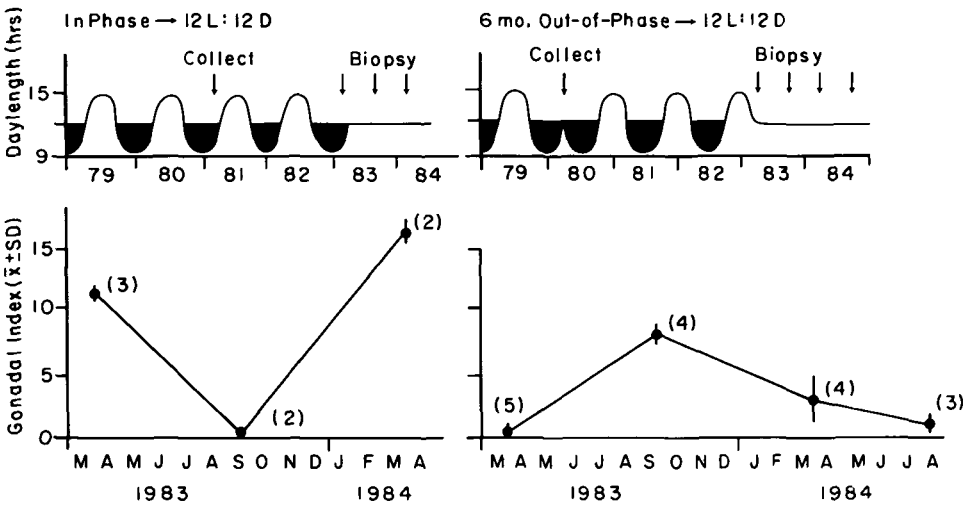

FIG. 8. Changes in gonadal indexes of *Pisaster ochraceus* reared for two to three years under seasonally changing photoperiods that were in phase or 6 mo out of phase with ambient photoperiods, then maintained under a fixed daylength of LD 12:12. Numbers in parentheses give number of animals surviving and sampled by biopsy on each date. Note temporal scales in top and bottom figures do not correspond.

cating that the persistence of the cycle in the absence of photoperiodic clues is not due to the animals following some other seasonally changing environmental factor (*e.g.*, slight seasonal changes in sea temperature). On the other hand, after about a year, the gametogenic cycles in animals entrained out of phase shifted into phase with in-phase animals. This suggests that the endogenous calendar, if present, was already set very early in life and is resistant to all but temporary entrainment.

DISCUSSION

The photoperiodic control system that we describe for gametogenesis in *Pisaster ochraceus* appears to be fundamentally different from that known to date for other systems. Even photoperiodism in the sea urchin *Strongylocentrotus purpuratus* seems fundamentally different from that in *P. ochraceus* (Pearse *et al.*, 1986), although both species share the same body plan (echinoderm) and the same habitat (rocky shallow-water of western North America). Moreover, both spawn gametes at about the same time (winter and spring) and share similar circannual rhythms (Halberg *et al.*, 1986). In *S. purpuratus* gametogenesis appears to require short daylengths of fall and winter to proceed, while in *P. ochraceus* gametogenesis is nearly or completely insensitive to short daylengths. Long daylengths of spring and summer apparently suppress gametogenesis in *S. purpuratus*, but lead to the initiation of a gametogenic cycle in the following 3 months in *P. ochraceus*. Finally, while there are experimental indications of an endogenous calendar underlying the gametogenic cycle of *P. ochraceus*, similar experiments with repeated "summers" and "winters" provide no evidence for such a calendar in *S. purpuratus*. However, experiments have not been done with *S. purpuratus* using fixed daylengths and we cannot yet say with any certainty that an endogenous calendar is not present.

Endogenous circannual rhythms or calendars are known to occur in a wide variety of animals (Saunders, 1982; Halberg *et al.*, 1983), and the presence of such a calendar under photoperiodic control in sea stars would not be unusual in itself. For example, Schwab (1971) showed photoperiodic control of testicular activity in starlings by observing that the testes remained large and spermatogenic when the animals were held at daylengths less than 11 hr, but they were always small and quiescent when held

at daylengths more than 13 hr. He further concluded that there was an endogenous circannual rhythm because the annual testicular cycle continued when the birds were held on a photoperiod of LD 12:12 (but see Hamner, 1971). In contrast, we found that the circannual gametogenic rhythm in *Pisaster ochraceus* continued unmodified at LD 15:9, 13:11, and 9:15, as well as at 12:12; the gametogenic rhythm could be phase-shifted only when the seasonal photoperiod rhythm was shifted, either 3 mo or 6 mo out of phase.

The gametogenic cycle in *Pisaster ochraceus* seems similar to the circannual rhythm of ovarian growth in the catfish *Heteropneustes fossilis*; that rhythm persists for over a year at constant temperature and continuous light, continuous darkness, LD 14:10, 12:12, and 9:15 (Sundararaj *et al.*, 1973, 1982). However, no attempt has yet been made to alter the rhythm by using seasonally changing daylengths as done with *P. ochraceus,* and there is no evidence of photoperiodic control of the rhythm. Such manipulation, indicative of photoperiodic control, has been done with the cycle of antler shedding and regeneration in deer (Goss, 1969*a, b,* 1980; Goss *et al.*, 1974). Like the gametogenic cycle in *P. ochraceus,* the antler growth cycle can be shifted 6 mo out of phase simply by shifting the seasonal light cycle 6 mo out of phase. Moreover, the antler shedding cycle is maintained under fixed photoperiods of LD 8:16, 13:11, 16:8, and 24:0. On the other hand, unlike the gametogenic cycle in *P. ochraceus,* the antler shedding cycle is obliterated at fixed daylengths between LD 12:12 and 13:11 (Goss, 1969*b*) and this is due to the 12-hr duration of the light or dark periods (Goss, 1984). Goss *et al.* (1974) found that they could increase or decrease the frequency of the antler growth cycle by changing the frequency of the changing light cycles; it would be valuable to determine whether the frequency of the gametogenic cycle in *P. ochraceus* could be similarly modified.

Olive and Garwood (1983) showed that when individuals of the polychete *Nereis diversicolor* are maintained under constant temperatures and "static" daylength regimes, they become gravid at different times separated by about 240 days. These observations were interpreted as evidence for a long-term endogenous rhythm that is "gated" and allows maturation to proceed at particular times after birth. Environmental factors such as light and temperature would act mainly to modify the maturation time before or after the gate is passed. Our finding that the gametogenic cycle of *Pisaster ochraceus* remains unaltered at different fixed photoperiods is evidence for the presence of such a long-term endogenous rhythm or calendar. Moreover, because the gametogenic cycle was evident in animals reared from juveniles that were held under a 6-mo out-of-phase photoperiodic regime for one year (Fig. 7), the rhythm apparently is set early in life. The presence of such an innate rhythm should be tested by rearing animals resulting from spawnings 6 mo apart. However, Olive and Garwood's model does not explain how the gametogenic cycle can be shifted as much as 6 mo out of phase simply by shifting the phase relationship of the annual photoperiodic cycle.

We were only able to shift the gametogenic cycle of *Pisaster ochraceus* out of phase by using an out-of-phase photoperiodic regime of seasonally changing daylengths; continuous exposure to either long or short daylengths had little or no effect on the overall expression of the cycle. Still, it would be premature to conclude that critical daylengths are not involved in regulating the photoperiodic response in *P. ochraceus.* Several species of neuropteran insects, for example, must experience a change from a critical short daylength to a critical long daylength to avert or terminate diapause (Tauber and Tauber, 1982). Our seasonal switching experiments (Fig. 1) indicate that long daylengths of spring and summer lead to the initiation of gametogenesis 6 mo out of phase with ambient, and critical long daylengths may be involved. In support of this suggestion we succeeded in initiating an out-of-phase gametogenic cycle by exposing animals to LD 15:9 for one month in midwinter and then maintaining them under LD 9:15 (although the animals later reverted to an

in-phase cycle) (Fig. 6). Finally, females exposed to continuous light regimes of LD 15:9 or 13:11 beginning in December contained vitellogenic oocytes the following August (Fig. 4), indicating a precocious initiation of gametogenesis. It is tempting to propose that some critical long daylength can initiate gametogenesis out of phase, and that long daylength needs to be followed by some critical short daylength for gametogenesis to continue. The problem with this proposal, however, is our finding that the gametogenic cycle continues in phase even when the spring-summer long daylengths were repeated (2 "summers," Fig. 1), or when the animals are maintained continuously on LD 15:9 or 13:11 (Fig. 4).

Currently there are two main models proposed to explain photoperiodic phenomena (Saunders, 1982). The "hour-glass" model proposes that some critical substance is produced or degraded over a critical period of light or dark, while the "circadian periodicity" or "Bunning's hypothesis" model proposes that a circadian oscillator measures daylength. Pittendrigh (1981*a*) reviewed the evidence for these models and concluded that most or all cases of photoperiodism can be explained by the latter model. This conclusion was reinforced by Pittendrigh *et al.* (1984), while Veerman and Vaz Nunes (1984) developed an "hour-glass time-oscillator counter" model that accounted remarkably well for photoperiodic induction of diapause in spider mites. If the photoperiodic response of the gametogenic cycle in *Pisaster ochraceus* is indeed insensitive to fixed daylengths, it (and the antler growth cycle) cannot be readily accounted for by either of these models. Rather, our experiments suggest that a new or much modified existing model is needed that incorporates changing daylengths and/or several different critical daylengths. As Goss *et al.* (1974) concluded 10 years ago: "In our present state of ignorance, we can only continue to gather data on this intriguing problem and hope that some day the bewildering profusion of facts may fall into place." Strange as it seems, sea stars appear to have joined deer in displaying a perplexing form of photoperiodism. Because sea stars and deer are so different in so many ways, this form of photoperiodism may be widespread and merits careful attention.

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