Marine Biology 67, 121–125 (1982)

MARINE BIOLOGY

Photoperiodic Regulation of Gametogenesis and Gonadal Growth in the Sea Star *Pisaster ochraceus*

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Abstract

A photoperiod regime 6 mo out of phase between December 1978 and August 1980 resulted in gametogenesis, gonadal growth, and spawning in laboratory-maintained sea stars [*Pisaster ochraceus* (Brandt)] 6 mo out of phase with individuals in the field or laboratory on normal celestial photoperiods at Santa Cruz, California (USA). The seasonal fluctuation of pyloric cecum size also was shifted when the sea stars were held on the out-of-phase photoperiod regime. Phase shift of all these events was evident within 6 to 9 mo. Long daylengths (or short nightlengths) in spring and summer apparently synchronize or entrain the initiation of gametogenesis and gonadal growth in fall.

Introduction

Most marine animals, like most terrestrial ones, have seasonal reproductive cycles. However, environmental variables that synchronize reproduction of marine animals rarely have been identified. Temperature changes have been shown experimentally to synchronize reproduction of some species that live in areas with seasonally changing sea temperatures. Consequently, the importance of seasonally changing sea temperatures has been emphasized in discussions of reproductive cycles of marine animals (Giese and Pearse, 1974). Nevertheless, many species display marked reproductive seasonality in areas where sea temperatures do not undergo large seasonal changes (e.g. the west coast of North America). Seasonally changing photoperiods, which are known to synchronize reproduction of many terrestrial plants (Vince-Prue, 1975) and animals (Farner and Follett, 1979; Beck, 1980), have only recently been shown experimentally to have a clear role in synchronizing gametogenesis in marine invertebrates, including a few marine molluscs (Richard, 1971; Price, 1979) and polychetes (Garwood, 1980) (see Segal, 1970, for review of earlier work). We report here experiments demonstrating photoperiodic control of gametogenesis and gonadal growth in the intertidal sea star *Pisaster ochraceus* (Brandt). This species has a well-synchronized reproductive cycle with spring spawning (Farmanfarmaian *et al.*, 1958; Mauzey, 1966; Nimitz, 1971), and over 20 yr ago Giese (1959) proposed that its reproduction might be under photoperiodic control.

Materials and Methods

All specimens of *Pisaster ochraceus* (Brandt) used in this study were collected from a mussel-covered, intertidal platform at Santa Cruz, California, USA. The largest individuals were preferentially collected, averaging about 250 g wet weight. Field individuals were collected and dissected at approximately 6 wk intervals from December 1978 through November 1980. Two collections were made for experimental sea stars. On 28 December 1978, 88 sea stars were collected and divided equally between two adjacent, light-tight rooms equipped with fluorescent lights (G. E. F40D Daylight) controlled by astronomic time switches (R. W. Cramer & Co., Type SY Model SOL). In both rooms, the sea stars were held in tanks with running seawater under identical conditions, except for photoperiod. In one room, the lights went on at local sunrise and off at local sunset (in phase or ambient photoperiod regime). In the other room, the lights were set to be 6 mo out of phase with ambient daily photoperiod (i.e., the longest day of the year was set for 22 December and the shortest day was set for 22 June).

By June, 1979, all the sea stars in the field had spawned all their gametes and had very small gonads (see Fig. 1). On 26 June 1979, 40 additional sea stars were collected from the field and divided equally between the two laboratory rooms. These individuals were held in tanks adjacent to those holding the sea stars collected in December 1978.

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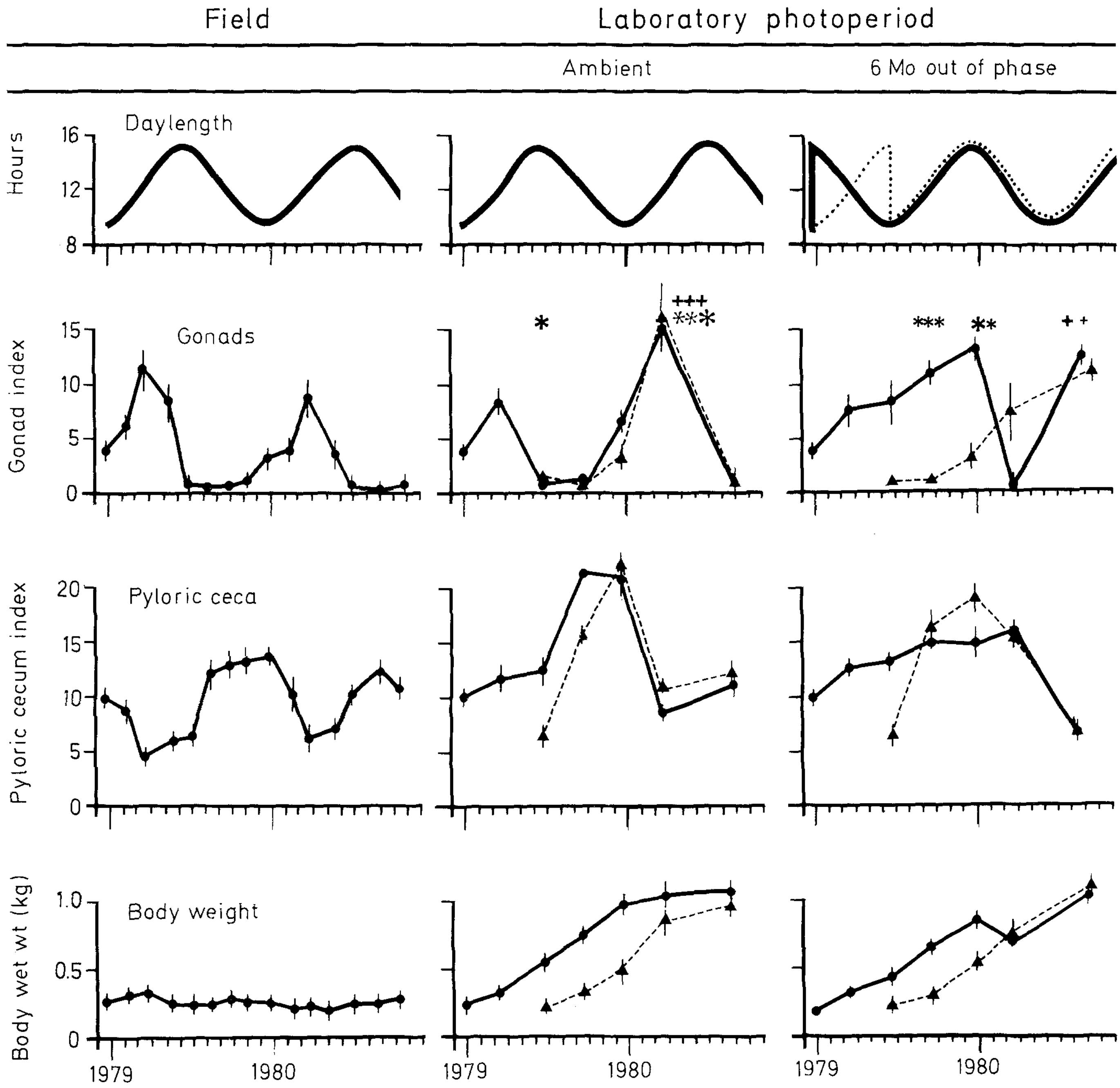

Fig. 1. *Pisaster ochraceus.* Changes in daylength, gonad index, pyloric cecum index, and total body wet wt in sea stars sampled from the field and from laboratory populations held under ambient (in-phase) and 6 mo out-of-phase photoperiod regimes, December 1978 to August 1980. Values are means ±1 SE. There were no differences between values for males and females in any of the samples. Continuous lines: individuals collected and maintained from December 1978; dashed lines: individuals collected and maintained from June 1979; Asterisks and crosses: laboratory observations of spawning by individuals collected in December 1978 and June 1979, respectively

Field and laboratory seawater temperatures were indistinguishable, and ranged from 10° to 14 °C in winter and spring and 13° to 17 °C in late summer and fall. Continuous supplies of mussels were provided as food. All the sea stars were weighed every 3 mo. Daily observations revealed no consistent behavioral differences between the individuals held in the two rooms, and there was no mid-winter quiescent period of moving and feeding as noted for field populations in Puget Sound, Washington (USA), by Mauzey (1966). Spawning was noted when observed. Males spawned white streams of sperms that left the tanks cloudy for hours; females spawned salmon-pink eggs that settled on the floor of the tanks and remained there for days. Because of our frequent observations, we probably recorded most or all spawning events of our laboratory sea stars.

Subsamples (4 to 10 individuals) were taken for dissection from each laboratory population on a quarterly schedule, and from the field population (10 individuals on each occasion) every 6 wk. The gonads and pyloric ceca were removed from each sea star, wet-weighed (not blotted), and organ indexes determined as the percent of the total body wet weight. Pieces of gonads from each sea star were fixed in Bouin's solution, embedded in paraffin, sectioned at 7 to 10 μm, and stained with Harris' hematoxylin, eosin Y, and orange G.

Results

All the sea stars held in the laboratory increased in body size, regardless of the photoperiod regime (Fig. 1). Feder (1970) found similar growth rates in well-fed laboratory specimens of *Pisaster ochraceus*. Unlike the laboratory individuals, adults in the field had similar weights in all samples. Feeding in the field, and therefore growth, was probably limited by periodic exposure of the sea stars during low tides, and by their need to hold firm when swept by large waves during high tides.

Changes in the gonadal sizes of laboratory individuals maintained on the ambient photoperiod regime were similar to those of local field sea stars (Fig. 1) and other populations of this species (Farmanfarmaian *et al.*, 1958; Giese, 1959; Mauzey, 1966; Nimitz, 1971). Gonadal growth, reflecting gametogenesis (Fig. 2: *1a–2a*), occurred between September and March when daylengths were less than 12 h. The gonads decreased in size, reflecting spawning, between March and June, when daylengths exceeded 12 h. Individuals of *Pisaster ochraceus* held in the laboratory on the ambient photoperiod regime spawned enormous numbers of gametes on 22 June 1979 and, after minor spawnings in April and May 1980, spawned massively on 29 June 1980.

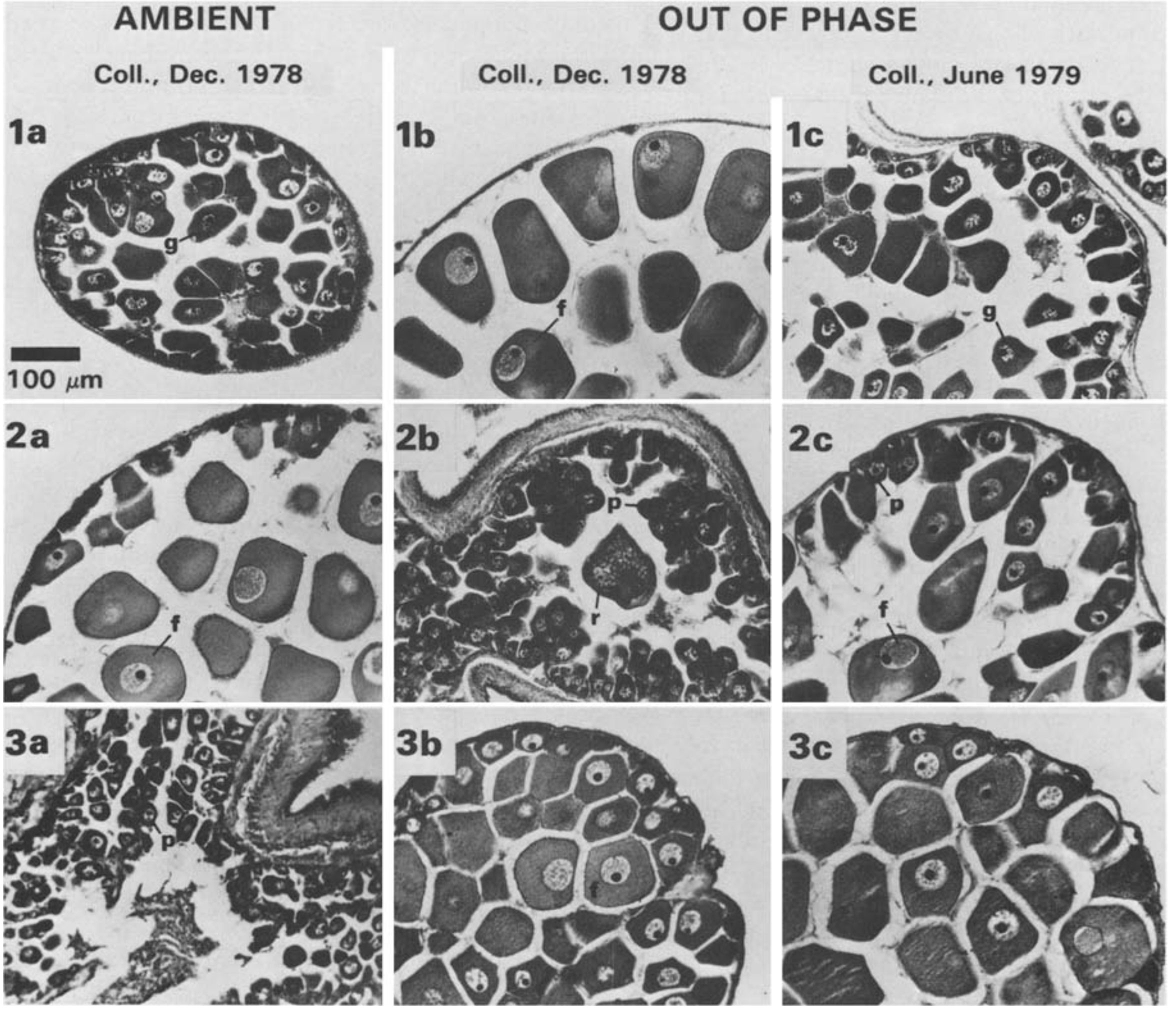

Fig. 2. *Pisaster ochraceus.* Photomicrographs of histological sections of ovaries taken from sea stars collected on 28 December 1978 and 26 June 1979 and held under ambient (a) and 6 mo out-of-phase (b and c) photoperiod regimes to 22 December 1979 (1a, b, c), to 25 March 1980 (2a, b, c), and to 23 August 1980 (3a, b, c). f, full-grown oocytes; g, growing oocytes; p, previtellogenic oocytes; r, relic oocyte. All photomicrographs at same magnification

Conversely, in sea stars collected in December 1978 and maintained on the photoperiod regime 6 mo out of phase, the gonads continued to grow after March 1979 and were largest in December 1979 (Fig. 1), when they were filled with sperms or full-grown oocytes (Fig. 2: *1b*). These individuals spawned small numbers of gametes in August and September 1979, and then underwent a massive spawnout on 30–31 December 1979, 6 mo after spawning by the individuals maintained on the ambient photoperiod regime.

In March 1980, when the gonads of individuals under ambient photoperiods (both in the field and laboratory) were large and filled with gametes, those of the out-of-phase individuals collected in December 1978 were very small (Fig. 1), and contained mainly spermatocytes or small previtellogenic oocytes (Fig. 2: *2b*). In the out-of-phase sea stars collected in 1978, the gonads began growing after March 1980 and, by late August 1980, were again large and full of sperms or full-grown oocytes (Fig. 2: *3b*); thus, the annual reproductive cycle of these individuals had completely shifted 180°. Unseasonal gametogenesis and gonadal growth between June and September 1979, and lack of pyloric cecal growth at that time (see below), indicate that the phase shift had begun within 6 mo after the shifting of the photoperiod cycle.

In the individuals of *Pisaster ochraceus* collected in June 1979, after spawning in the field, and maintained at photoperiods 6 mo out of phase, the gonads began growth after September (Fig. 1), in synchrony with the in-phase individuals, but by March 1980 the ovaries contained *both* full-grown oocytes and numerous previtellogenic oocytes (Fig. 2: *2c*). Although many of the sea stars spawned in July and August, they all still had large gonads full of gametes in late August 1980 when they were dissected. The annual reproductive cycles in these individuals had been shifted 180° within 9 mo after the photoperiod cycle was shifted.

The pyloric ceca of sea stars are nutrient storage organs, and in many species, including *Pisaster ochraceus,* their size fluctuates inversely with changes in the size of the gonads (for review see Harrold and Pearse, 1980). The size of the pyloric ceca of both field individuals and those held under the ambient photoperiod regime fluctuated inversely with the size of the gonads (Fig. 1); cecum indexes decreased markedly from December 1979 to March 1980, when gonadal growth was greatest, and then increased again by August 1980, when the gonads had reached their smallest size. By contrast, in the out-of-phase individuals collected in December 1978, the size of the pyloric ceca varied little until after March 1980. In particular, ceca did not increase in size between June and December 1979 when the gonads showed unseasonal growth; subsequently, ceca shrank between March and late August 1980 when the gonads grew markedly. Changes in size of the pyloric ceca of the out-of-phase sea stars collected in June 1979 followed those of individuals held under the ambient photoperiod regime until December 1979; after March 1980 their changes were consistent with the out-of-phase pattern.

Discussion

The effect of the out-of-phase photoperiod regime on individuals of *Pisaster ochraceus* collected in December 1978 was evident by June 1979. This phase shift could have been a response either to the unseasonal long daylengths (or short nightlengths) between December and March, or to the unseasonal short daylengths (or long nightlengths) between March and June. The out-of-phase individuals collected in June 1979 experienced unseasonal short days between June and September, but the normal cycle of gonadal growth still began in September 1979 and was completed by about March 1980; the gametogenic cycle in these individuals must have been synchronized or entrained before they were collected in June. We suggest that long spring and summer daylengths (or short nightlengths) normally synchronize or entrain the gametogenic cycle, and that the unseasonal gametogenesis and gonadal growth in individuals collected in December 1978 resulted from the unseasonal long daylengths given them between December 1978 and March 1979. The normal pattern of gametogenesis and gonadal growth shown by sea stars collected in June 1979 might have been synchronized by the seasonal long daylengths they experienced between March and June before they were collected.

Although long daylengths probably synchronize the initiation of gametogenesis and gonadal growth, continued growth may be enhanced by the short daylengths experienced during the normal period of gonadal growth. Gonadal size in March 1980 was lower in those individuals collected in June 1979 and held in the out-of-phase room (6 mo of long days) than in those held in the ambient room (6 mo of short days). These observations are in agreement with experiments reported by Greenfield (1959), showing that gonadal growth was inhibited in continuous light and enhanced in near continuous darkness in specimens of *Pisaster ochraceus* held between November and January.

Photoperiodic control of reproduction in other animals involves photoreceptors and intricate systems of hormonal synchronization. Many sea stars, including *Pisaster ochraceus,* have a complex ocellus at the tip of each ray. These ocelli are photosensitive, as shown by behavioral and electronmicroscopic studies (Yoshida and Ohtsuki, 1966; Brandenburger and Eakin, 1980; Penn and Alexander, 1980). However, sea stars also are photosensitive over most or all of their body surface (Yoshida, 1966, 1979). Moreover, endocrine systems in echinoderms are poorly understood. A radial nerve polypeptide initiates meiosis and spawning in sea stars (Kanatani, 1979; Schuetz and Glad, 1981), but it is not known to have any role in regulating early gametogenesis or gonadal growth. Steroids have been isolated from sea star organs, and their levels in the gonads fluctuate seasonally, being highest near the time when gonadal growth begins; these steroids have been experimentally shown to enhance gonadal growth (Greenfield, 1959) and oocyte growth (Schoenmakers, 1981). Whether biosynthesis of such steroids is regulated by mechanisms under photoperiodic control, what such mechanisms are, and where they are located within the sea stars, all remain to be elucidated.

Acknowledgements. Critical reading and comments on the manuscript by R. A. Cameron, K. K. Davis, A. C. Giese, D. R. Lindberg, and V. B. Pearse are gratefully acknowledged. We are also indebted to R. W. Pierce for providing the astronomic time switches, B. Steele for daily care of the sea stars, and W. T. Doyle, Director of the Center for Coastal Marine Studies, for funding and logistic support at the Joseph M. Long Marine Laboratory.

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Date of final manuscript acceptance: December 10, 1981.
Communicated by N. D. Holland, La Jolla