REPRODUCTIVE PERIODICITIES OF INDO-PACIFIC INVERTEBRATES IN THE GULF OF SUEZ. I. THE ECHINOIDS *PRIONOCIDARIS BACULOSA* (LAMARCK) AND *LOVENIA ELONGATA* (GRAY)

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ABSTRACT

Cellular changes in the gonads of the echinoids, *Prionocidaris baculosa* (Cidaroidea) and *Lovenia elongata* (Spatangoidea), in the Gulf of Suez are followed over a year. Gametogenesis leading to spawning begins in synchrony among individuals of *P. baculosa* in April, and spawn-out occurs in July and August. Gametogenesis is not as synchronous among individuals of *L. elongata*; it begins in mid- and late winter, and the prolonged spawning period occurs mainly between April and September. Nutritive phagocytic tissue is prominent in the gonads of both species, its globulation changing over the year, especially in *L. elongata*. The nutritive phagocytes are involved in gametogenesis by phagocytizing gametogenic cells during part of the year and nourishing them when gametogenesis is leading toward spawning. Fluctuations in photoperiod, salinity, and sea temperature do not seem to be directly related to the synchronization of reproduction, but accumulation of nutrients, perhaps indirectly related to the effects of sea temperature and photoperiod, may have importance in synchronization.

INTRODUCTION

Situated between 27° and 30° north latitude, the Gulf of Suez is well above the Tropic of Cancer. It is also a long, narrow, shallow body of water, about 300 km long, 40 km wide, and only about 35 m in average depth (Anonymous, 1922; Gohar, 1954). The prevailing winds blow out of the north-northwest. They drive the surface waters south toward the adjacent Red Sea, and cause considerable evaporation, cooling, and sinking (Mohamed, 1940; Gohar, 1954; Neumann & McGill, 1962). The relatively northern position, the shallow depth, and the prevailing northerly winds cause the Gulf of Suez to be temperate in character, and seasonal changes of the same magnitude as in the eastern Mediterranean occur in such factors as photoperiod and sea temperature (Oren, 1962).

On the other hand, the fauna and flora of the Gulf of Suez are tropical. Except for the nearly insignificant Suez Canal, the gulf has been connected only to the Red Sea since the beginning of the Pleistocene (Gohar, 1954), and probably was entirely above sea level during the last ice age (Sewell, 1948:475). Almost all the species in the Gulf of Suez, therefore, are relatively recent arrivals from the Red Sea. The present fauna and flora of the Red Sea, in turn, probably entered from the Indian Ocean after the Plio-

cene, perhaps mainly since the last glacial period (Sewell, 1948). Many species that are found in the Gulf of Suez consequently occur also in other parts of the Indo-Pacific region, through its equatorial area as far south as South Africa and Australia; these species may occur as far east as the central Pacific.

The Gulf of Suez, relatively temperate yet inhabited by typically tropical species, is therefore of unusual ecological and physiological interest. Environmental changes, such as those of sea temperature, must be near the extremes experienced by most of the species present. Indeed, there are only poorly developed small fringing reefs in the gulf, and many of these are dead, while coral reefs flourish in the adjacent Red Sea (Crossland, 1938). Many species typical of Indo-Pacific shores, such as the intertidal ophiuroid, *Ophiocoma scolopendrina*, and the echinoid, *Tripneustes gratilla*, are abundant at the mouth of the Gulf of Suez at Al-Ghardaqa, yet are absent or very rare at least on the western shores of the gulf. Moreover, the mangrove, *Avicennia marina*, is fairly common south of the Gulf of Suez to Mozambique but absent in the gulf itself, and the abundant halophyte along the shores of the gulf, *Halocnemon strobilaceum*, is replaced farther south by the ecologically comparable species, *Arthocnemon glaucum* (Kassas & Zahran, 1967).

Reproduction of temperate marine species is usually limited to periods when temperature and food are favorable (Giese, 1959). Reproduction of tropical species is poorly understood, however, because, in strictly tropical areas, environmental factors should be uniform and always favorable for reproduction, yet many species do have reproductive periodicities (e.g., Mortensen, 1921, 1938; Thorson, 1950; Giese, 1959). As pointed out elsewhere (Pearse, 1968), this dilemma perhaps can be resolved by realizing that (1) most tropical areas are not seasonally equable environments, and (2) most tropical species reported to have seasonal reproductive periods were studied in areas where seasonal environmental fluctuations, including those of sea temperature, definitely occur.

Because of the extreme conditions in the Gulf of Suez, environmental effects on reproduction should be most pronounced there. This was suggested earlier by Mortensen (1938), who was perplexed by the indications of reproductive periodicities he found at the mouth of the gulf. The present study was initiated to delineate the reproductive periodicities at a cellular level of many of the common species in the gulf and, if possible, relate these periodicities to the environmental fluctuations that occur. Results on two echinoids, the cidaroid *Prionocidaris baculosa* forma *typica* (Lamarck) and the spatangoid *Lovenia elongata* (Gray), are presented in this paper. These two species are both relatively widespread in the Indo-Pacific region; *P. baculosa* occurs south to western South Africa and east to the Philippines (Mortensen, 1928) and Japan (Utinomi, 1954), and *L. elongata* occurs

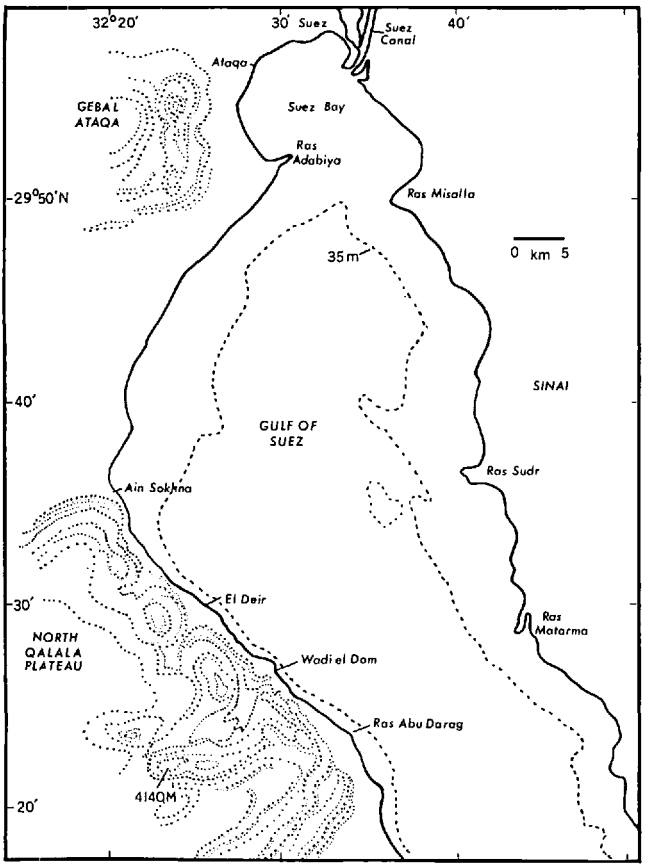

FIGURE 1. Map showing the location of the collecting site (Wadi el Dom) and other features of the northern third of the Gulf of Suez.

south to Mozambique and east to at least Japan and eastern Australia (Mortensen, 1951).

MATERIALS AND METHODS

Both species were collected from a site on the northwestern shore of the Gulf of Suez (Fig. 1). The site was at a prominent small delta of land formed at the mouth of Wadi Quseb, a major drainage system of the North Qalala Plateau, which rises steeply from the coast. The eastern shore of this delta, at Wadi el Dom (29°26'N, 32°30'E), provides a small sheltered cove where both species occurred in abundance. Fringing the shore between low tide level and about 2 m depth was a narrow coral reef where

most specimens of *P. baculosa* occurred. Specimens of *L. elongata* were found one to two hundred meters offshore, burrowing in a sand bar that was exposed during the spring tides. Specimens of *P. baculosa* also often were found walking on the surface of the sand bar. Although other areas along the western shore of the Gulf of Suez were examined, particularly along the coastline of the North Qalala Plateau between Ain Sokhna and Ras Abu Darag (Fig. 1), no other large population of either species was found, even in adjacent coves.

Both species were collected while swimming with snorkel and face mask. Specimens of *P. baculosa* were simply picked off the bottom or reef, while those of *L. elongata* were found by raking the sand with the fingers, an iron bar, or a garden rake. On exceptionally calm days individuals of *L. elongata* could be detected by characteristic oval depressions in the sand, but usually waves disturbed the sand enough to erase all traces of the animals.

Samples of *P. baculosa* were collected in 1965 on August 22, October 24, November 21, and December 24; and in 1966 on January 16, February 12, March 6, April 2 and 24, May 29, June 24, July 19, August 15, and September 13. Samples of *L. elongata* were taken in 1966 on March 6, April 1 and 23, May 29, June 25, July 19, September 12, October 16, and December 4; and in 1967 on January 22. All the animals in the samples were of a sexually mature size, and their sizes varied little among the samples. Test diameter of specimens of *P. baculosa* ranged from 33 to 69 mm, averaging 56.6 mm; test length of specimens of *L. elongata*, measured from within the frontal notch, ranged from 40 to 76 mm, averaging 61.4 mm.

Individuals of both species were very numerous when sampling began, and the samples could be collected within a few minutes. However, individuals of both populations became very scarce before the year of sampling was completed. The population decrease was perhaps due to sampling pressure, but other factors might have been involved. Individuals of *P. baculosa* first became difficult to find in May, 1966, after 10 months of sampling and when a total of 73 animals had been collected. The animals seemed more abundant on the sand bar in April than previously; perhaps they were wandering into deeper water offshore. In June, July, and August they were very scarce, and the animals of the August and the final September samples were all that could be found in over an hour's search. Only one animal was found in October, 1966, although they were abundant the year before. Three animals were seen on the sand bar in December, 1966, and in January, 1967, many were on the sand bar, perhaps wandering into shallower waters. By March and April, 1967, individuals of *P. baculosa* were again abundant on both the sand bar and the reef.

Individuals of *L. elongata* were first difficult to find in June and July, 1966, and those that were found were usually together in groups of two or three animals. Many dead specimens were found in the sand bar in Au-

gust, most with their spines still intact. Over an hour's search yielded only one live animal, and this one had minute gonads and diseased bare areas on the test. Perhaps the population was undergoing a mass mortality similar to that described for *Mellita quinquiesperforata* by Salsman & Tolbert (1965). Clean empty tests were abundant in the sand in September, while living animals were difficult to find. Twelve empty tests were collected and measured. Their length ranged from 48 to 72 mm, averaging 61.9 mm, while the 14 live animals of the sample ranged in length from 53 to 70 mm, averaging 63.0 mm. The October, December, and January samples were found only after long searches. Specimens of the October sample were clumped together in small areas seemingly indistinguishable from surrounding areas, but the individuals of the December and January samples were widely scattered. No specimens were found in February and March, 1967, although they were very abundant the previous year.

Test size of each animal was measured with calipers soon after the samples were collected. The gonads were then removed and their general condition noted. One gonad of *L. elongata* and the oral tip of one gonad of *P. baculosa* were fixed in Bouin's solution, embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin. Occasionally several parts of different gonads in the same animal were fixed together; as in most other echinoids, their histological condition was identical.

Periodically all the animals in a sample and their gonads were weighed, and gonadal indices calculated by the formula: gonadal weight times 100 divided by total animal weight. The guts of the specimens of *P. baculosa*, from the beginning of the esophagus to the end of the rectum and washed free of contents, were also weighed, and gut indices were calculated.

Histological analyses of the ovaries were done as previously described (Pearse, 1965). Fifty oocytes or ova were selected at random and grouped into size classes of 17 μ each, from 0 μ upwards, and frequency polygons of the size classes were plotted. Only oocytes showing a nucleolus in section, or ova showing a nucleus, were selected. The thickness of the layer of nutritive phagocytes in the ovaries of specimens of *P. baculosa* was estimated by direct measurement of transverse sections. In the ovaries of specimens of *L. elongata*, however, the nutritive phagocytes did not form a discrete layer, and their amount was estimated subjectively as little, moderate, or much.

A method similar to Holland's (1967) was used to analyze the testes. The thicknesses of the layers of the spermatogenic cells (spermatogonia, spermatocytes, and spermatids), nutritive phagocytes, and spermatozoa were measured in typical testicular lobes with approximate radii, in transverse section, of 210 μ in *P. baculosa* and 336 μ in *L. elongata.* The thicknesses of the different cell layers varied considerably within the lobes of some

testes, and measurements from several different places were often needed to obtain accurate estimates.

Sea-surface temperatures at the shore were measured about every hour from sunrise to late afternoon on the days of the collections, and maximum and minimum sea temperatures were estimated from these measurements. After August, 1966, a maximum-minimum thermometer was secured to the bottom (*ca.* 1-2 m) near the reef for over a day. Measurements from the maximum-minimum thermometer corresponded to within 1°C of those estimated from the hourly surface measurements.

Results

Prionocidaris baculosa (Lamarck).—The inner coelomic surfaces of the gonads of *P. baculosa* were usually covered with a thick layer of fibrous connective tissue. Tough mesenteric strands firmly anchored the fibrous covering, and the gonads underneath, to the test and both loops of the intestine. Running down the center of each gonad, perpendicular to the test, was a peculiar, stiff, keel-like structure. The gonads themselves were very spiny because of the many calcareous spicules they contained. Their color was generally dull, being whitish, grey, tan, ochre, or brown, with no relation to sex. No seasonal change was observed in these general aspects of the gonads.

Very little oozing occurred when the gonads were cut, and usually only nutritive phagocytes were in the little oozing that did occur. In late June, July, and August, however, ripe gametes oozed from the cut gonads of many animals (Fig. 2, A). Mature sperm were white, while ova were pale grey. This was the only time the animals could be sexed in the field without a microscope, and spawning was undoubtedly restricted to this period.

The gonadal indices showed a general increase from December to late June, and a decrease from late June to mid-September (Fig. 2, A) (by analysis of variance, $F = 5.9685$, $P < 0.005$). The increase in gonadal indices between December and March was due to an increase in size or number of the nutritive phagocytes, because little gametogenesis occurred at that time. The decrease in gonadal size occurred when mature gametes were numerous, and was due to spawn-out.

The gut indices were highest in the spring and lowest in the summer, although these differences were slight; the average, one standard deviation, and the range for each date of sampling were: 24 Dec., 2.3 ± 0.5, 1.7-3.2; 2 Apr., 2.6 ± 0.5, 1.8-3.3; 24 June, 1.7 ± 0.4, 0.9-2.1; and 13 Sept., 1.7 ± 0.6, 1.3-2.7 (by analysis of variance, $F = 6.1570$; $P < 0.005$). This slight seasonal fluctuation in the gut indices was similar to, but not as pronounced as, that found with *Strongylocentrotus purpuratus* (Lawrence *et al.*, 1965). The maximum just preceded the onset of gametogenesis and the minimum occurred during spawn-out (see Figure 2 and text below).

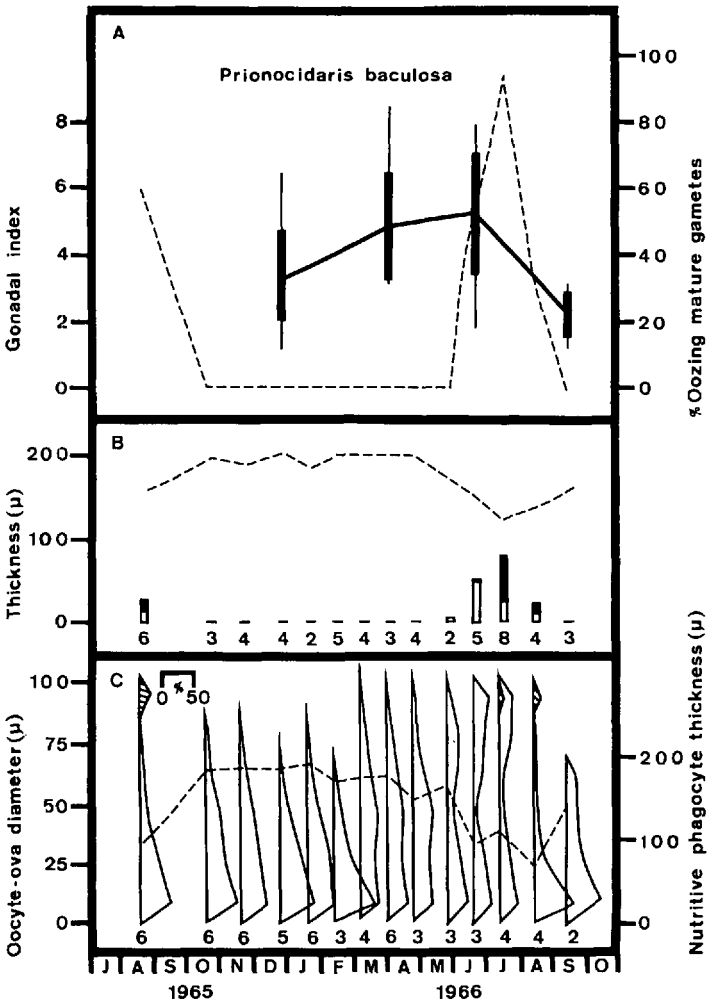

FIGURE 2. Reproductive changes in *Prionocidaris baculosa* at Wadi el Dom, Gulf of Suez: A, means, standard deviations, and ranges for gonadal indices, and the percentage of animals oozing mature gametes upon dissection (dashed line); B, averaged thicknesses of the spermatogenic (open bars), spermatozoal (solid bars), and nutritive phagocytic (dashed line) layers in typical transverse sections of the testes, about 210 μ in radius (number of males in each sample is given beneath each histogram); C, averaged frequency polygons of different sized oocytes and ova (hatched), and average thicknesses of the nutritive phagocytic layer (dashed line) in the ovaries (number of females in each sample is given beneath each frequency polygon).

Predominant in the guts of most animals were pieces of coral, either smooth and worn or freshly chipped. Many of the coral pieces were from dead material; the urchins probably were digesting the epiflora and fauna on them. However, live coral was also ingested, and slimy remains of the coral tissues adhered to the pieces in the gut. Many animals were collected from the surfaces of both live and dead coral that they were ingesting. The guts also contained a wide variety of other materials, including silt, crustacean and molluscan shells, greenish masses of plant material, and chips of wood. With such an omnivorous diet, the amount of food available to the animals probably did not fluctuate much over the year.

During most of the year the testes of *P. baculosa* were filled with nutritive phagocytes (Fig. 2, B). The plasma membranes of these cells were not distinct, and usually there was simply a solid mass of nutritive phagocytic tissue in the testes (Fig. 3). The tissue was very vacuolated, and it often contained many inclusions, including basophilic, eosinophilic, and golden-brown globules of diverse shapes and ranging in size from about 1 μ to 15 μ in diameter (Fig. 3). There was much variability in the number of globules present in the testes of different animals, and there was little seasonal pattern except that all types seemed most numerous in July and August. Often a large genital sinus occurred, particularly in the fall after spawning and when the germinal layer seemed shrunken and pulled away from the peritoneum (Fig. 3). The genital sinus usually contained numerous spherulated cells that were full of strongly staining eosinophilic globules. During the fall, the peritoneum itself contained many small (*ca.* 5 μ), golden-brown globules.

A few spermatocytes, spermatids, and spermatozoa were scattered among the nutritive phagocytic tissue throughout the year; these probably were being phagocytized by the nutritive phagocytes. Spermatogonia were scattered along the base of the germinal layer at all times during the year. Small nests of spermatogonia, with several spermatogonia in each, occurred at the base of the germinal layer in the testes of one of three males in the sample collected on April 2 (Fig. 3). By the end of May, spermatocytes formed a discrete layer. All the males of the June sample had a thick layer of spermatocytes, and one male had a few spermatozoa clustered in the lumen. A discrete layer of spermatids was never found, and spermatogenesis probably proceeded quickly from spermatocytes to spermatozoa. Very ripe animals occurred only in the July sample, and in August most of the males had completed spawning and relict spermatozoa and spermatogenic cells were being phagocytized by the nutritive phagocytes. Even though spawning occurred only during July and August, it probably was not a very synchronized spawn-out, because the males in these samples were in different stages of maturity (Fig. 4).

Nutritive phagocytes were also abundant in the ovaries, usually com-

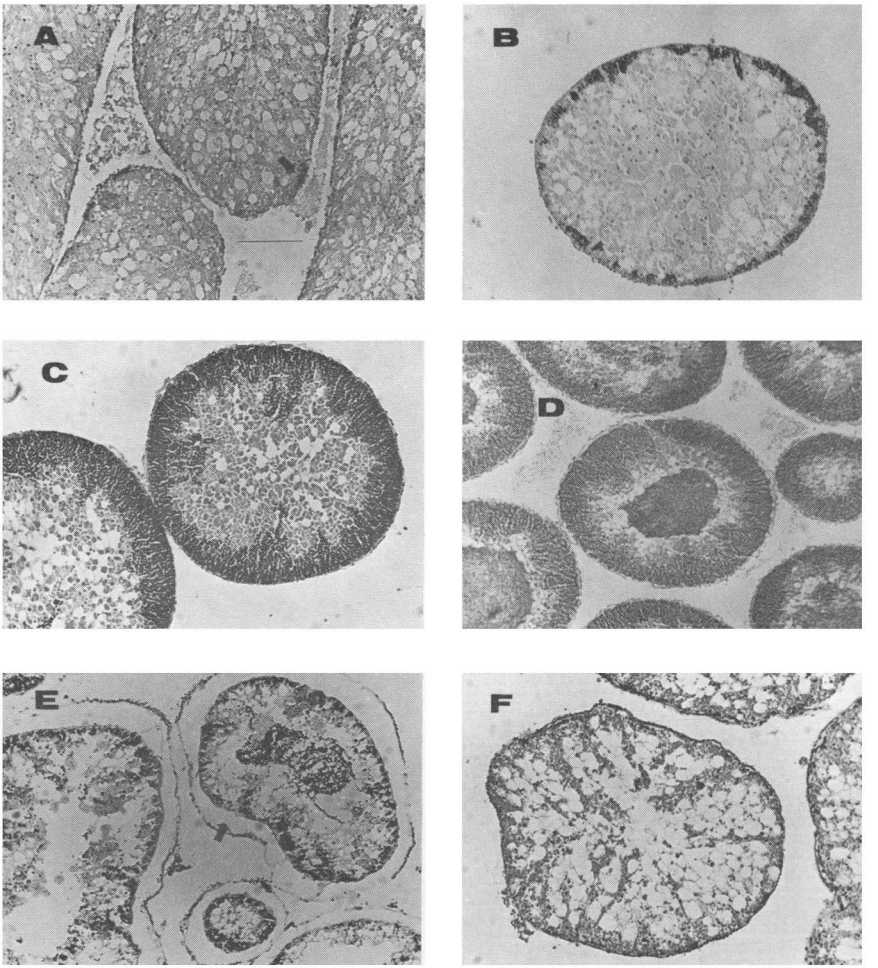

FIGURE 3. Histological sections of the testes of *Prionocidaris baculosa*: A, April 2, small nests of spermatogonia (arrow); B, May 29, thin continuous layer of spermatocytes; C, June 24, thick continuous layer of spermatocytes and nutritive phagocytic tissue especially rich in eosinophilic globules; D, July 19, nearly ripe, with the lumen full of spermatozoa; E, August 22, recently spawned and with relict spermatozoa; note the large genital sinus (arrow); F, November 21, increase in the nutritive phagocytic tissue. Some of these individuals are depicted as histograms in Figure 4. Scale in A is 100 μ; all are at the same magnification.

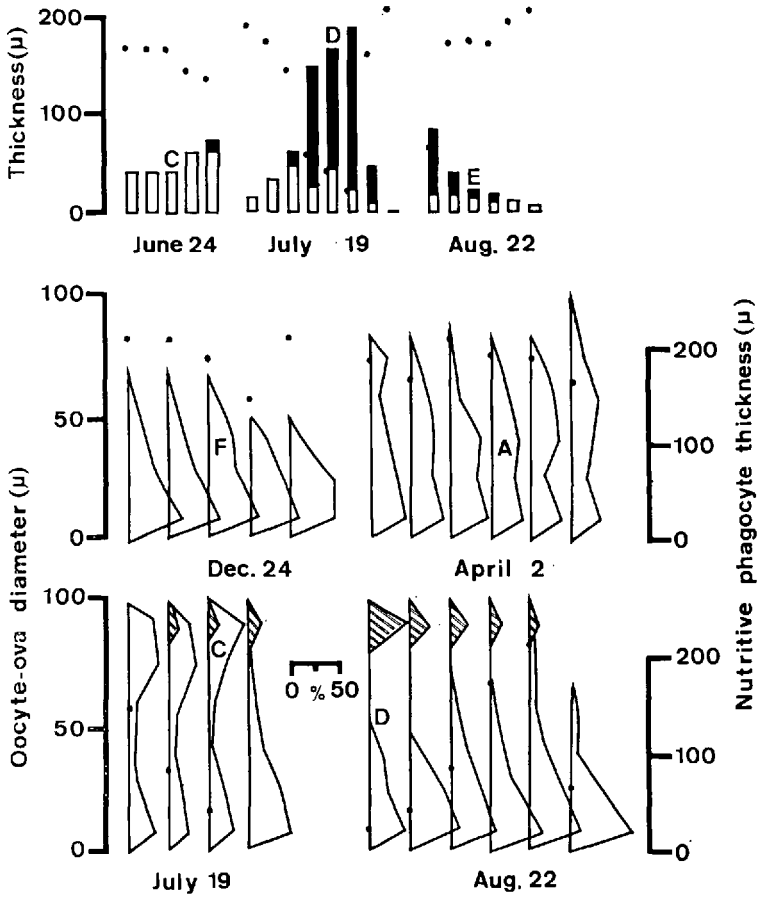

FIGURE 4. Data for each of the individuals in several samples of *Prionocidaris baculosa*. Testes are represented as histograms and ovaries as size-frequency polygons of oocytes and ova as in Figure 2, but the thicknesses of the nutritive phagocytic tissue are represented by dots. Letters correspond to individuals whose gonads are shown in the photomicrographs in Figures 3 and 5.

pletely filling them. There was not as much nutritive phagocytic tissue between May and September as in the other months of the year, however, because during this period oocytes were abundant, forming a more or less continuous layer along the base of the germinal epithelium, and crowding out other cells. During the other parts of the year the oocytes were scattered and scarce. In August, after spawn-out, an empty lumen was usually present (Fig. 5). As in the testes, the ovarian nutritive phagocytes usually contained numerous eosinophilic, basophilic, and golden-brown globules.

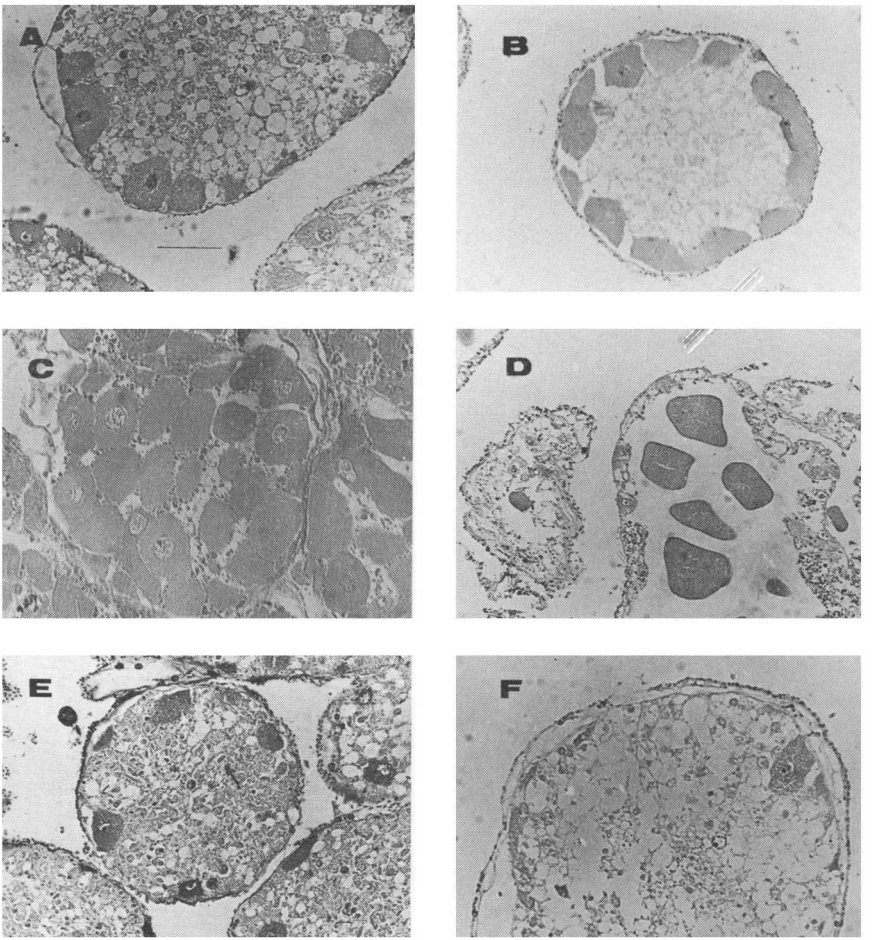

FIGURE 5. Histological sections of the ovaries of *Prionocidaris baculosa*: A, April 2; B, May 29, ripening, with a continuous layer of growing oocytes; C, July 19, nearly ripe, and full of full-grown oocytes; D, August 22, recently spawned and with relict ova; E, November 21, abundant eosinophilic globules in the nutritive phagocytic tissue, some being rectangular in shape (arrow); F, December 24. Some of these individuals are depicted as oocyte-ova size-frequency polygons in Figure 4. Scale in A is 100 μ; all are at the same magnification.

The eosinophilic globules were abundant in all the samples, and often they were large and rectangular (Fig. 5), up to about 10 μ by 20 μ, and had a fibrous structure. The basophilic and golden-brown globules were most abundant between October and May, being particularly abundant in April.

A large genital sinus often was present also, especially in the fall after spawning, and it contained many spherulated cells full of eosinophilic globules.

Oocytes up to about 60 μ in diameter were found in all the ovaries sampled (Figs. 2, C; 5). The cytoplasm of the oocytes of all sizes tended to be weakly eosinophilic, and there was no distinct, small basophilic stage, as found in some other echinoids (e.g., Holland, 1967). Many or most of the oocytes in the ovaries sampled between August and March were in various stages of disintegration. They were surrounded by nutritive phagocytes, and were probably being phagocytized. During this period oogenic growth was apparently always terminated by phagocytosis. In the March and April samples less disintegration of oocytes occurred, and more medium-sized oocytes were found. There was an almost continuous layer of medium-sized and large oocytes along the ovarian walls in late May (Fig. 5), and in June and July most of the oocytes for the year's spawning season were fully grown. In two of the four females of the July sample a few oocytes had matured into ova; this probably just preceded spawning (Fig. 4). Ova in another female of the July sample, and in the females of both August samples were few, and were probably the relicts of an earlier spawn.

Oogenic growth stages in the different individuals of each sample were very similar (Fig. 4), indicative of close oogenic synchrony among the different individuals. Moreover, there appeared to be only one oogenic growth cycle each year that culminated with spawning. The oogenic cycle took about 2 months to complete (from mid-April to mid-June). However, as shown in the July sample in Figure 4, spawning did not occur in synchrony among all the animals, because maturation had not begun in one female, had just begun in two others, and was completed and most ova spawned in the fourth. As in the males, mature gametes probably accumulate and are released at different times in different animals during the July-August spawning period.

Lovenia elongata (Gray).—The four tufted gonads of *L. elongata* were suspended by thin mesenteries from the aboral surface of the test, and each had a prominent gonoduct leading to its gonopore. Both ovaries and testes were usually various shades of orange or ochre, and there was little or no seasonal change in color. Most of the males in all the samples had gonoducts full of spermatozoa that oozed copiously upon dissection. Although numerous spermatozoa were present all year, a distinct seasonality of spermatogenesis occurred, as shown in Figure 6, B and discussed below. Ova were never numerous enough to ooze from the cut ovaries; in fact, ova were never seen. In Figure 6, A, the percentage of animals oozing gametes therefore reflects only the males with numerous spermatozoa in their gonoducts.

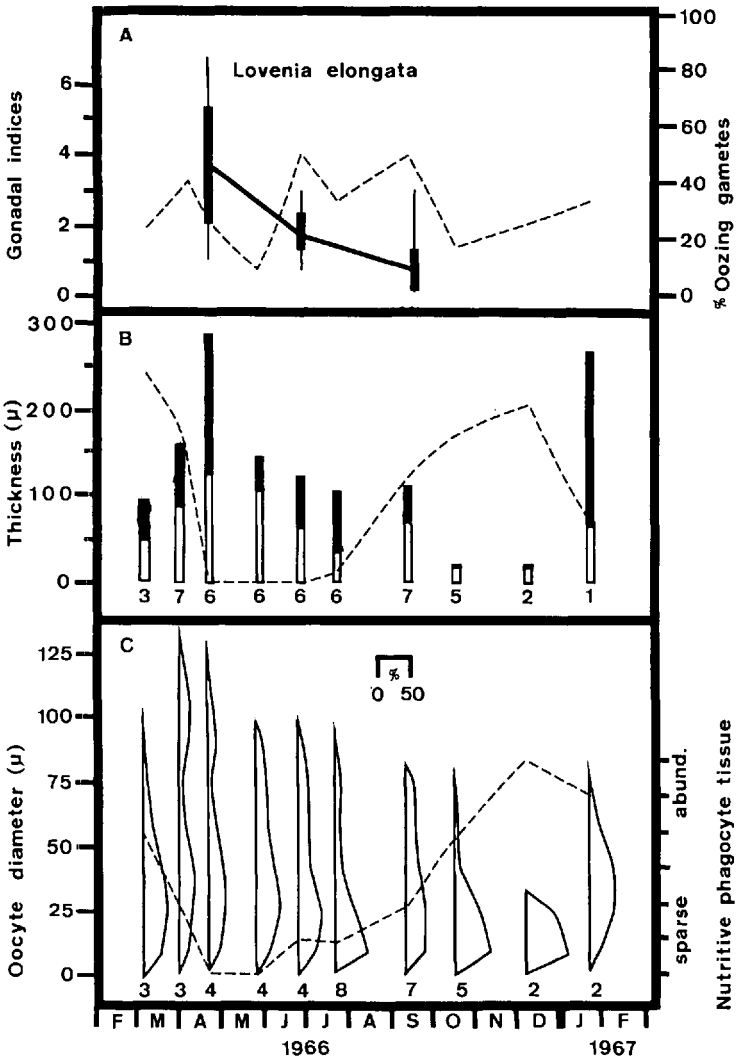

FIGURE 6. Reproductive changes in *Lovenia elongata* at Wadi el Dom, Gulf of Suez (presented as in Fig. 2): A, gonadal indices and percentages of animals oozing numerous gametes upon dissection (dashed line); only males oozed numerous mature gametes; B, thicknesses of the spermatogenic cells (open bars), spermatozoal (solid bars) and nutritive phagocytic (dashed line) layers in typical transverse sections of the testes, about 336 μ in radius; C, size-frequency polygons of the oocytes, and average thicknesses of the nutritive phagocytic layer (dashed line) in the ovaries.

Between late April and mid-September, the gonadal indices fell (Fig. 6, A) (by analysis of variance, $P < 0.001$). This decrease was probably due completely to spawning because there was little nutritive phagocytic tissue present during this period. Indeed, the nutritive phagocytic tissue increased between mid-June and mid-September (Fig. 6, B, C), while the gonadal indices continued to decrease.

Gut indices were not determined for specimens of *L. elongata*, because the gut walls were extremely thin and delicate, and the guts themselves were always full of sand. The gut tissue broke up when attempts were made to wash the sand free. The amount of sand in the gut did not seem to differ over the year.

Spermatogenesis occurred mainly in the winter and spring, with the layer of spermatogenic cells being thickest in April and May (Figs. 6, B; 7). The spermatids usually formed a discrete layer, 5 μ to 10 μ thick, distal to the spermatocyte layer. As spermatozoa accumulated in the lumen, the amount of nutritive phagocytic tissue decreased, until in May and June it had almost completely disappeared. From late April to September the number of spermatozoa decreased, and much of the testicular lumen was an empty, cell-free space; spawning therefore occurred primarily during this time.

Toward the end of the spawning period, in the July and September samples, the amount of nutritive phagocytic tissue increased in the testes. This tissue was phagocytizing both spermatozoa and spermatogenic cells, and it was filled with small basophilic globules (Fig. 7) that probably consisted mainly of the nuclear material of the gametes. The nutritive phagocytic tissue was particularly rich with basophilic globules in the October sample, but eosinophilic globules were also more evident. In the males of the December and January samples the eosinophilic globules had become extremely abundant, while the basophilic globules were much less prominent. The eosinophilic globules were presumably degradation products of gametes as well as new nutrient stores. As spermatogenesis proceeded in the winter and spring, all the globules decreased; they were probably being used to nourish the new spermatogenic cells. The nutritive phagocytes in the animals of the March and April samples were almost completely deglobulated.

As shown in Figure 8, the testes were only imperfectly synchronized among the males of each sample. Most of the stages of spermatogenesis were present in the sample taken on April 1,—from much nutritive phagocytic tissue and no spermatozoa, to very little nutritive phagocytic tissue and many spermatozoa. Various stages of maturity and spawn-out were represented in the June, July, and September samples.

The single male of the January sample had more spermatozoa than might be expected from the annual pattern established by the other samples (Fig. 6, B). Because of the variability shown within samples (Fig. 8), this ani-

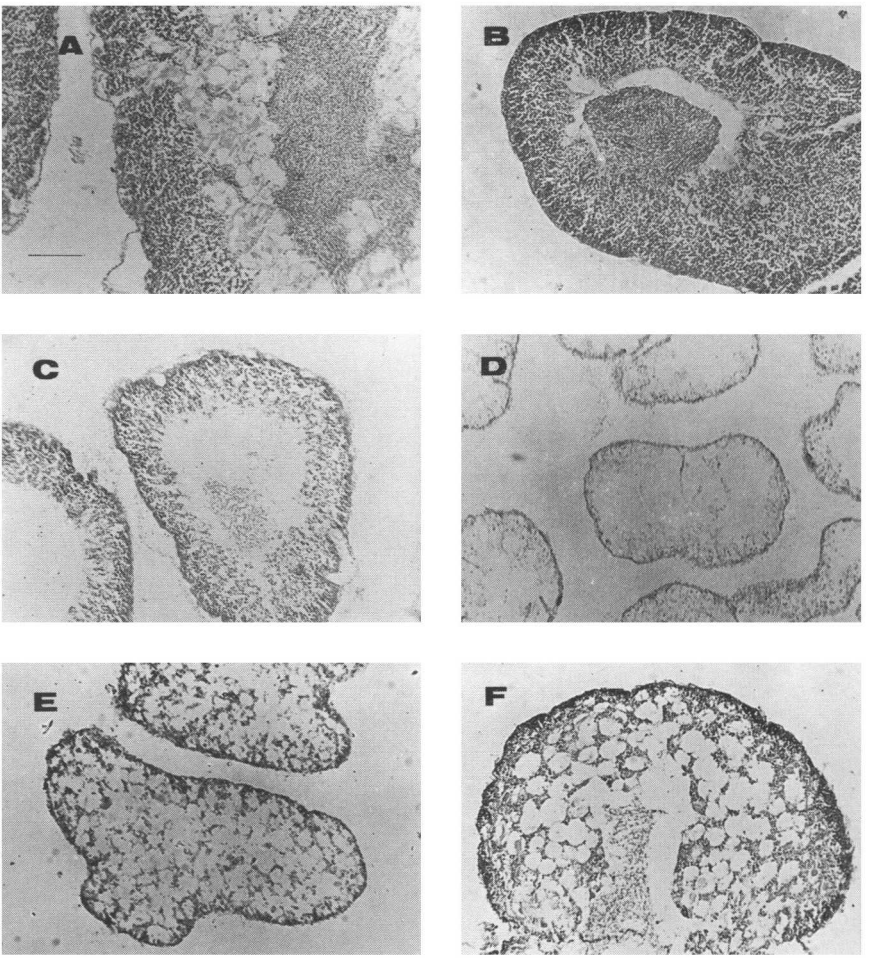

FIGURE 7. Histological sections of the testes of *Lovenia elongata*: A, April 1, layers of spermatogenic cells and deglobulated nutritive phagocytes, and a lumen full of spermatozoa; B, April 1, layer of spermatogenic cells and a lumen full of spermatozoa, but no nutritive phagocytic layer; C, July 19, layer of spermatogenic cells, only a few spermatozoa in the lumen, and no nutritive phagocytic layer; D, July 19, completely spawned, with an empty lumen and a thin layer of nutritive phagocytes; E, October 16, extensive growth of nutritive phagocytic tissue, and many small basophilic globules; F, December 4, extensive growth of nutritive phagocytic tissue, both eosinophilic and small basophilic globules, and a small amount of spermatozoa in the lumen. All of these individuals are depicted as histograms in Figure 8. Scale in A is 100 μ; all are at the same magnification.

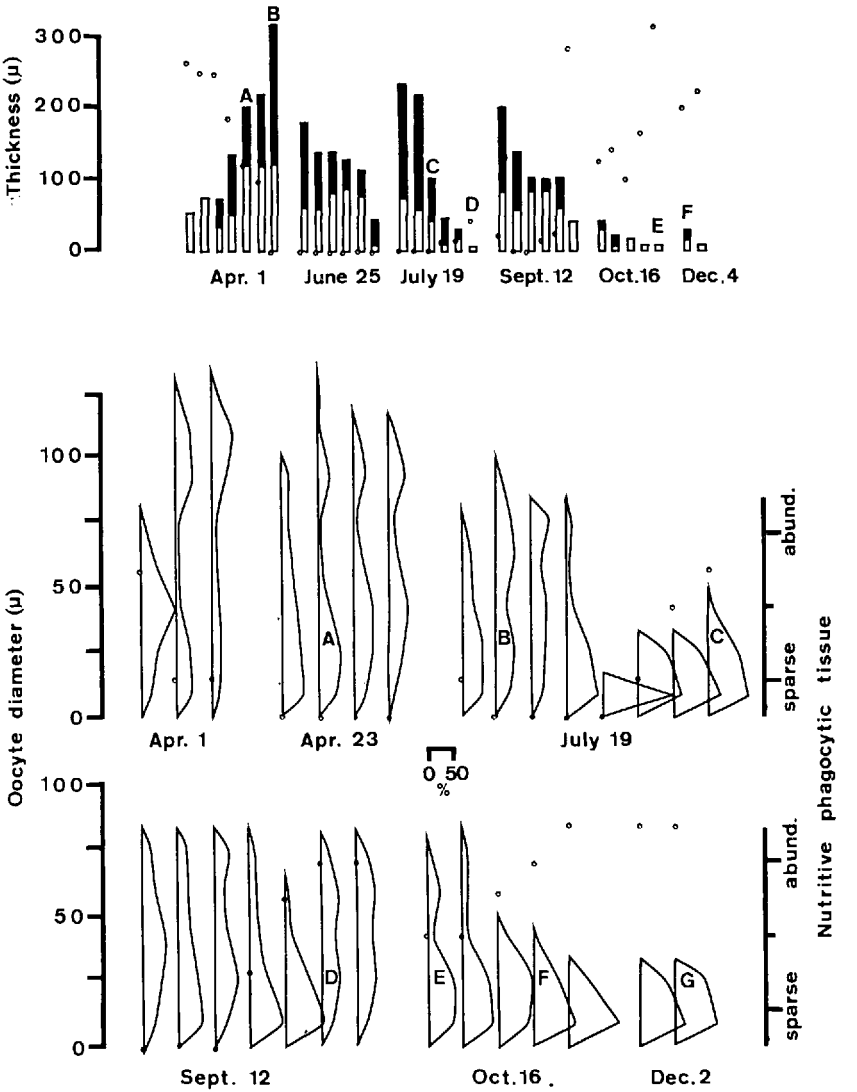

FIGURE 8. Data for each of the individuals in several samples of *Lovenia elongata*. Testes represented as histograms, ovaries as size-frequency polygons of oocytes, and the thicknesses of the nutritive phagocytes by dots, as in Figure 4. Letters correspond to individuals whose gonads are shown in the photomicrographs of Figures 7 and 9.

mal probably can be considered as atypical and unusually precocious for the time of its collection.

Growth of oocytes occurred mainly in the winter and spring, with full-grown oocytes being numerous in the April samples (Fig. 6, C). The change from small basophilic oocytes to large eosinophilic oocytes was gradual, occurring when the oocytes were about 50 μ in diameter. Full-grown oocytes were not found after April; spawning apparently began in late April and, after that time until about September, oocytes were spawned as soon as they had completed growth. Ova were not seen in either the histological or fresh preparations so it is not known whether maturational divisions occurred just prior to, or after, spawning. There certainly was no prolonged storage of ova as occurs in many other echinoids. Disintegrating and phagocytized oocytes of all sizes became increasingly abundant after June, reaching a peak in the October and December samples. As in *P. baculosa*, therefore, termination of the period of oogenic growth was associated with an increased phagocytic activity of the nutritive phagocytes.

The nutritive phagocytes in the ovaries were very diffuse and did not form a distinct layer whose thickness could be easily estimated. In the April and May samples, in particular, the nutritive phagocytes formed distinct thin follicles around the larger oocytes (Fig. 9). Many of the oocytes were attached by stalks to the follicular walls. Although not directly measurable, the amount of nutritive phagocytic tissue obviously varied seasonally, as indicated in Figures 6, C and 9, with an increase occurring in the fall and winter, followed by a decrease in the spring. Changes in the globulation of the ovarian nutritive phagocytes paralleled those in the testes. Basophilic globules were most abundant in the samples taken from June to October when oocytic disintegration and phagocytosis were at their heights. The basophilic globules were replaced by eosinophilic globules, and these became very abundant in December and January. The ovarian nutritive phagocytes were almost completely deglobulated in the samples of March, April, and May. Golden-brown globules, similar to those in *P. baculosa*, were also present in the ovaries, especially in those of the June, July, and September samples.

As in the males, there was a good deal of variability among the females of each sample (Fig. 8). The larger oocytes in half of the females of the July sample probably would have continued their growth and would have been spawned, but in the other females the nutritive phagocytic tissue had increased, and the larger oocytes were being destroyed (Fig. 9). The variation in the October sample probably reflects various stages of oocyte destruction, and not active oogenesis.

Sea Temperatures.—Sea temperatures fluctuated seasonally at the collecting site by about 10°C; an average of about 18°C occurred between early Janu-

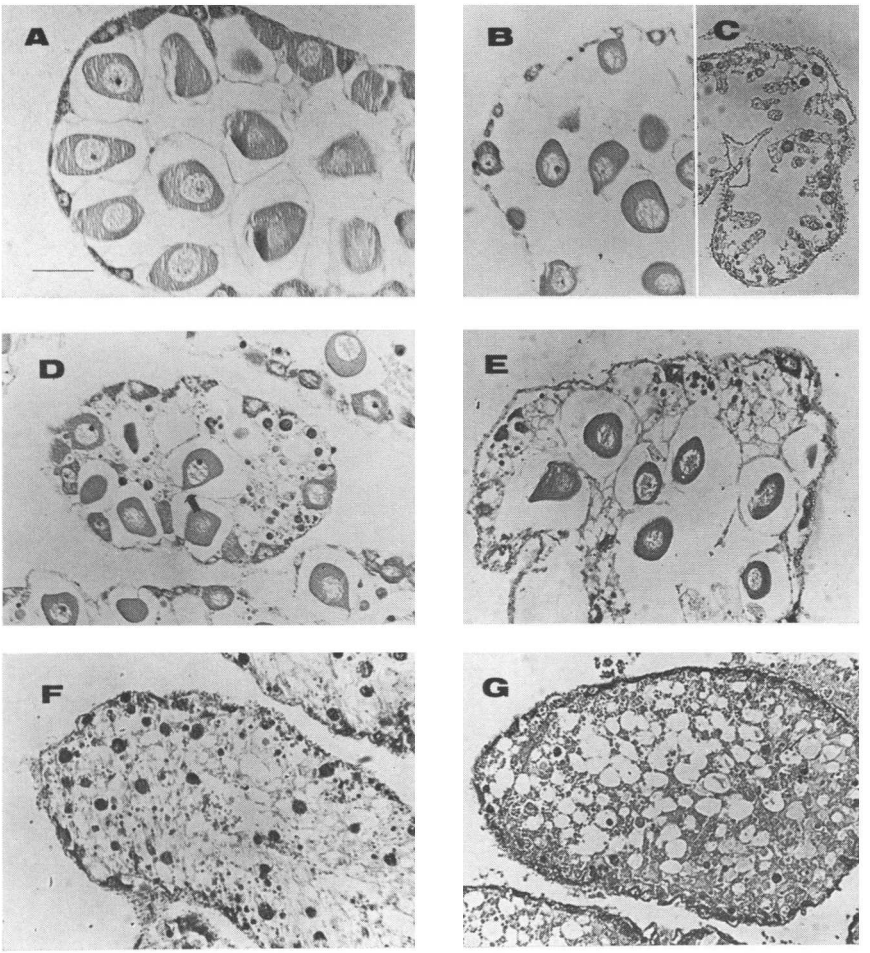

FIGURE 9. Histological sections of the ovaries of *Lovenia elongata*: A, April 23, large oocytes in follicles formed by the thin nutritive phagocytes; B, July 19, oocytes and very little nutritive phagocytic tissue; C, July 19, spawned, showing ingrowing nutritive phagocytic tissue; D, September 12, large oocytes in follicles and basophilic globules in the nutritive phagocytes; note the stalk attaching the oocyte to the follicular wall (arrow); E, October 16, large oocytes in follicles and many basophilic globules in the nutritive phagocytes; F, October 16, extensive growth of nutritive phagocytic tissue with many basophilic globules; small oocytes are being disintegrated; G, December 4, extensive growth of nutritive phagocytic tissue and abundance of both eosinophilic and basophilic globules. All of these individuals are depicted as size-frequency polygons of oocytes in Figure 8. Scale in A is 100 μ; all are at the same magnification.

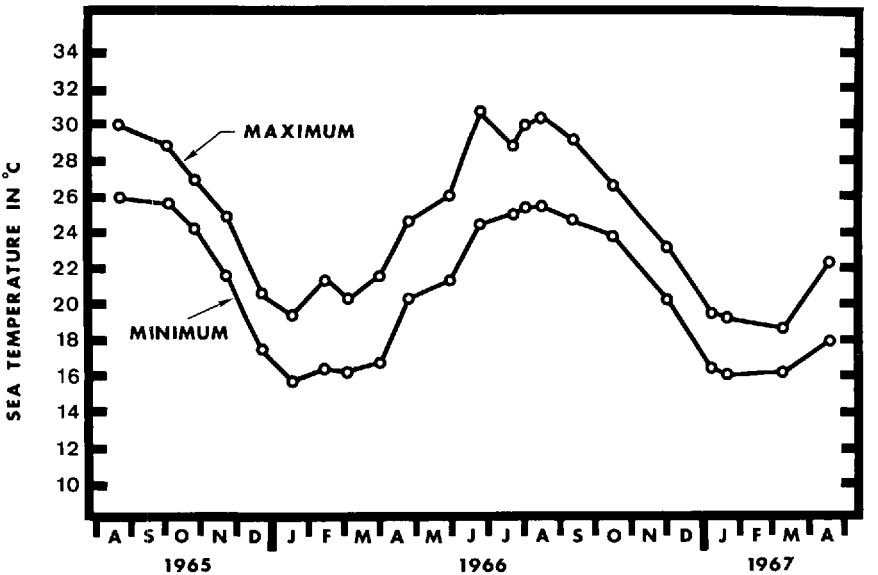

FIGURE 10. Changes in maximum and minimum surface temperatures of the sea at the sampling site at Wadi el Dom, Gulf of Suez, between August, 1965, and April, 1967.

ary and early April, and an average of about 27°C occurred between mid-June and late September (Fig. 10). Daily fluctuations in sea temperature were of the magnitude of 3° to 5°C. The low was always at sunrise; the high was in the early or midafternoon, depending on the tidal sequence. The fluctuations of sea temperature, both daily and seasonal, were similar to those of the air temperature. In the spring and summer, however, the maximum air temperatures were usually from 5° to 10°C higher than the sea temperatures; warming of the sea lagged behind that of the air in the spring and then was stabilized in the summer by evaporation caused especially by the northerly winds. The midday high tides (semidiurnal, range about 1 m) in the summer also prevented unusually high temperatures in the sea by bringing in cooler waters when solar warming was most intense.

Cloudy days and rain occurred rarely and probably had very little effect on sea temperature. When rain did occur, it was usually in the winter, and then only traces reached the ground. In the first two weeks of October, 1965, however, several centimeters of rain fell in a series of downpours. Reddish mud was washed out of the North Qalala Plateau, making the water at the sampling site extremely turbid and covering much of the reef with silt. Although some corals and other animals were killed by the silting,

little lasting damage occurred, and few traces of the silt or dead animals remained the following spring. Very little or no rain fell in the fall of 1966, as is more normal.

Discussion

Aspects of Gametogenesis.—*P. baculosa*, a cidaroid, and *L. elongata*, a spatangoid, are near the opposite ends of the phylogenetic spectrum of echinoids; cidaroids are the only extant members of the Paleozoic subclass Perischoechinoidea, and spatangoids are among the more recent and specialized members of the subclass Euechinoidea (Durham, 1966). That the process of gametogenesis is very similar in these two species indicates that it is a general one for echinoids. This process includes the occurrence of more or less uniform changes throughout all the lobes of the gonads of each animal, the fluctuating abundance of the nutritive phagocytes and their several types of globules, the gradual oocytic growth from small oocytes along the ovarian wall to the accumulation of full-grown oocytes in the lumen, and the accumulation and differentiation of spermatocytes along the testicular wall through spermatids to spermatozoa massed in the lumen. Previously, gametogenesis has been studied mostly in regular euechinoids (e.g., *Diadema setosum*, Yoshida, 1952; *Strongylocentrotus intermedius* and *S. nudus*, Fuji, 1960; *S. purpuratus*, Holland & Giese, 1965; *Sterechinus neumayeri*, Pearse & Giese, 1966), and in all these cases the process is similar to that observed in *P. baculosa* and *L. elongata.*

Holland (1967) has described gametogenesis in the cidaroid *Stylocidaris affinis*, the only other member of the Perischoechinoidea in which this has been done.[1] The first detailed description of gametogenesis in an echinoid, done by Caullery in 1925 with *Echinocardium cordatum*, is the only such work on a spatangoid. Gametogenesis in both of these species is similar to that described in the present paper; the involvement of, and changes in, the nutritive phagocytes are nearly identical in *E. cordatum* and *L. elongata.* Holland (1967) noted that ova are present only briefly in the ovaries of *S. affinis*; he suggested that this is a character of the primitive echinoderms and that prolonged storage of ova is a specialization unique to the euechinoids. In accordance with Holland's suggestion, prolonged storage of ova does not occur in the cidaroid, *P. baculosa.* However, it also does not occur in the spatangoid, *L. elongata*; indeed, ova were never found in the gonads of this species. Prolonged storage of ova also may not occur in the spatangoid, *Moira atropos*, because Moore & Lopez (1966) never found more than a few ova ("ova without nuclear membranes") in the ovaries of this species. Nevertheless, there is a prolonged storage period

[1] After this paper was accepted for publication, B. F. McPherson (Contributions to the biology of the sea urchin *Eucidaris tribuloides* (Lamarck). Bull. Mar. Sci., *18* (2): 400-443) described gametogenesis in another cidaroid; gametogenesis in *Eucidaris* appears to be very similar to that in *Prionocidaris.*

for ova in the ovaries of *E. cordatum* (Caullery, 1925; Moore, 1936); this species and *L. elongata* both belong to the family Loveniidae. If the lack of prolonged storage in cidaroids reflects a primitive character and prolonged storage reflects a specialization of euechinoids, as Holland (1967) suggested, then lack of storage of ova in some spatangoids is probably a secondary regressive character that has developed at the species level.

Holland (1967) also noted a prolonged period of accumulation of spermatocytes in the males of *Stylocidaris affinis*. He suggested that this character might be unique to the cidaroids among echinoderms. However, there was little or no prolonged period of accumulation in *P. baculosa*, and, as Holland cautioned, gametogenesis needs to be described for more species of echinoids before meaningful phylogenetic implications can be made.

The nutritive phagocytes in the gonads of both *P. baculosa* and *L. elongata* seem closely involved with gametogenesis in (1) destroying relict gametes and gametogenic cells at the close of the spawning period, (2) destroying gametogenic cells that form out of season, and (3) nourishing gametogenic cells that form during the reproductive period. It is not clear, however, whether the nutritive phagocytes destroy healthy, growing gametogenic cells, and thereby directly regulate gametogenesis by limiting the period when gametogenesis can be completed. The nutritive phagocytes may merely phagocytize already disintegrating gametogenic cells and thus have no direct regulatory role. Nutrients are probably simply stored, with little synthesis of new materials, because these cells seem remarkably free of mitochondria and endoplasmic reticula (Takashima & Takashima, 1965; Verhey & Moyer, 1967).

Nutritive phagocytes are abundant in the gonads of other echinoids, and they probably have a similar function in all echinoids. Seasonal changes in the globules of the nutritive phagocytes in at least *Stylocidaris affinis*, *Strongylocentrotus purpuratus*, and *Echinocardium cordatum* are very similar to those that occur in *L. elongata*. Phagocytosis by the nutritive phagocytes apparently does not begin in the ovaries of *E. cordatum* until the first oogenic cycle has been completed by spawning (Moore, 1936); after that time the nutritive phagocytes fluctuate more or less seasonally between their opposing functions (Caullery, 1925). Also in some asteroids, such as *Echinaster sepositus* (Cognetti & Delavault, 1960) and *Leptasterias hexactis* (Chia, 1968), nutritive phagocytes aid in destroying oocytes growing out of season and in nourishing them during the reproductive period. In another asteroid, *Odontaster validus*, however, there is almost never any nutritive phagocytic tissue in the ovaries, and oocytic disintegration and nutrient transfer occur independently of non-gametogenic cells (Pearse, 1965). The fact that nutritive phagocytes are not involved in gametogenesis in *O. validus* suggests that these cells do not directly regulate gametogenesis

even when they are present, but that they only facilitate destruction of gametes and transfer of nutrients.

Synchronization of the Reproductive Periods.—Mortensen (1938) found the individuals of *P. baculosa* near the mouth of the Gulf of Suez to be unripe in May and June, and full of mature gametes in late July; this is an indication that the reproductive periodicities described for this species occur year after year throughout the Gulf. Mortensen (1937) also found ripe specimens of *L. elongata* near the mouth of the Gulf of Suez in early May, and this finding corresponds well with those in the present paper. Work apparently has not been done on the reproduction of either of these species elsewhere in their Indo-Pacific distribution. In another Indo-Pacific echinoid, *Diadema setosum*, reproduction occurs in the boreal summer in the Gulf of Suez and off central Japan, probably throughout the year when near the equator, and in the austral summer on the Great Barrier Reef (Pearse, 1968). A similar general pattern of reproduction may exist in *P. baculosa* and *L. elongata*, with reproduction occurring in the austral summer off South Africa, and also off southeastern Australia in *L. elongata*. Only one oogenic growth cycle, taking about two months, occurs each year in the Gulf of Suez in at least *P. baculosa*; perhaps the number of oogenic growth cycles per year increases as the populations get closer to the equator.

In *Echinometra mathaei*, another Indo-Pacific echinoid, reproduction is restricted to the summer months in the Gulf of Suez and probably off central Japan, but is continuous elsewhere, including in the northern Red Sea and at the southern limits of its distribution off southwestern Australia (Pearse & Phillips, 1968). Because in the Gulf of Suez the reproductive periodicities of *L. elongata* are rather poorly defined, with a layer of spermatogenic cells and spermatozoa being present all year, perhaps this species has a general pattern of reproduction more similar to that of *E. mathaei* and reproduces continuously in most areas.

Both *P. baculosa* and *L. elongata* have planktotrophic larvae (Mortensen, 1937, 1938), and their summer spawning periods in the Gulf of Suez are probably adaptations insuring the larvae with both abundant planktonic food and temperatures favorable for development. Environmental synchronization of gametogenesis and spawning, however, remains little understood. Although seasonal fluctuations in sea temperature usually are considered the main environmental factor that synchronizes reproduction in marine animals (see Thorson, 1946, 1950; Giese, 1959), the pronounced seasonal fluctuations in sea temperature in the Gulf of Suez do not relate well to the observed reproductive periodicities. Gametogenesis in *L. elongata*, for example, begins in the mid-winter when the temperatures are below those that this species experiences in most other parts of its distribution. Spawning begins in late April, when the maximum sea temperatures

reach about 25°C, but it is completed in September before even the minimum sea temperatures drop much below this value. Gametogenesis leading to spawning in *P. baculosa* begins in about April, when the maximum sea temperatures are between about 22° and 25°C. Yet apparently only one gametogenic cycle is completed during the year although the sea temperatures continue to be "favorable" for gametogenesis for the following six months. Moreover, spawning in *P. baculosa* begins in July, nearly a month after the maximum sea temperatures of the year are reached.

Fluctuations in sea temperature and reproduction do seem to be well correlated for *Arbacia punctulata* off eastern North America and *Paracentrotus lividus* off western Europe (Harvey, 1956) as well as for *Echinocardium cordatum* off western Europe (Moore, 1966). These echinoids spawn during the summer in the northern parts of their distribution and during the winter in the southern parts, and their spawning periods correspond to supposed maximum and minimum temperature requirements. Minimum temperature requirements for reproduction also can be estimated from the geographical patterns of reproduction of some tropical species (e.g., Yonge, 1940; Pearse, 1968). However, when closely examined, as has been done for *P. baculosa* and *L. elongata*, most data show little direct relation between sea temperatures and reproduction. *Arbacia punctulata*, for example, seems to have a midwinter spawning period off Cape Cod (Boolootian, 1966). Boolootian (1966) also has reported that while sea temperatures might affect the amount of gonadal growth that occurs in *Strongylocentrotus purpuratus* off western North America, they do not correlate with the timing of reproduction. Some species, such as *Sterechinus neumayeri* (Pearse & Giese, 1966) and the asteroid *Odontaster validus* (Pearse, 1965, 1966) in the Antarctic and *Stylocidaris affinis* off Naples (Holland, 1967), have very discrete reproductive periodicities even though there is very little or no seasonal fluctuation in sea temperatures. Similar examples of little or no direct relation between fluctuations of the sea temperature and reproduction can be found for other groups of marine animals (see, for example, Galtsoff, 1961), and such a direct relationship may be exceptional rather than general ("Orton's Rule," Thorson, 1946) as formerly supposed.

Other environmental factors often thought to be important for the synchronization of reproductive periodicities in marine animals include seasonally changing photoperiods, salinities, and food (Giese, 1959). Photoperiods are probably not important for at least *L. elongata*, because its midwinter period of gametogenesis in the Gulf of Suez corresponds to a time when the photoperiod is shorter than occurs at any time in any other area of its distribution. Much the same argument can be made against the possible importance of fluctuations in salinity; the salinities in the northern part of the Gulf of Suez (about 42.5‰, Mohamed, 1940) are always higher

than elsewhere in the distribution of any of the species there. Moreover, with little rainfall occurring in the Gulf of Suez, and that occurring very sporadically, fluctuations in salinity are slight and irregular (Gohar, 1954) and probably could not be of importance for rhythmic environmental synchronization.

Fluctuations in food and accumulated food reserves may be more important for synchronizing reproductive periodicities in *P. baculosa* and *L. elongata*. Gametogenesis in both species begins after the accumulated reserves in the nutritive phagocytic tissue have reached the maximum, as indicated both by the thickness of the tissue and the gonadal indices. Perhaps a critical amount of nutrients in the nutrient phagocytes is needed before gametogenesis can proceed. The amount of nutrient reserves accumulated is a function of the amount of food available and the rates of both feeding and metabolism. These factors, in turn, all can be functions of temperature and photoperiod, and, in this way, temperature and light fluctuations could be of indirect importance for synchronizing reproduction. The relation of temperature to these factors may be complex and obscure, especially in the tropics. Moore and his colleagues (Moore & McPherson, 1965; Moore & López, 1966; Moore, 1966), for example, have found that the rate of growth in several tropical western Atlantic echinoids is highest at relatively low temperatures; apparently metabolism is so rapid at higher temperatures that little energy can be utilized for animal and presumably gonadal growth. Fuji (1962) found seasonal differences in the rates of feeding and assimilation in a temperate Japanese echinoid, which were related to sea temperatures; maximum total assimilation occurred during the spring period of gonadal growth, and the minimum occurred in September after spawn-out. Moreover, Farmanfarmaian & Giese (1963) found a marked increase in the respiratory rate of *Strongylocentrotus purpuratus* at relatively high temperatures, which would rapidly exhaust any nutrient reserves. Corresponding to this observation, gonadal growth in *S. purpuratus*, as determined by gonadal indices, is much lower in the warmer areas of its distribution than in the cooler areas (Boolootian, 1966). Accumulation of nutrients in *P. baculosa* and *L. elongata* during the fall and winter, therefore, may be partly due to a decrease in temperature that lowers metabolic demands more than it does the assimilation rate. Moreover, gametogenesis may perhaps be terminated in midsummer in both species because the food supply is inadequate to maintain gametogenesis while the rates of respiration and activity are high.

This hypothesis relating nutrient reserves, and indirectly temperature, to reproduction is a very tentative one, and several problems with it are evident. Most obvious is the fact that both *P. baculosa* and *L. elongata* also occur along the equator where the sea temperatures are always relatively high, and yet where at least some mature individuals probably occur at all

times of the year. Perhaps: (1) Compensation is made whereby a lower "critical level" of nutrients is needed to initiate gametogenesis than in the Gulf of Suez, and fewer gametes are produced by any one gametogenic cycle. (2) The time needed to accumulate nutrients to a "critical level" is longer than in the Gulf of Suez so that a complete gametogenic cycle takes much longer in any one individual, but the individuals are so asynchronous with each other that some mature ones can always be found.

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Sumario

Periodicidades Reproductivas de Invertebrados Indo-Pacificos en el Golfo de Suez. I. Los Equinoideos *Prionocidaris baculosa* (Lamarck) y *Lovenia elongata* (Gray)

Se describen cambios reproductivos al nivel celular en dos equinoideos, *Prionocidaris baculosa* (Cidaroidea) y *Lovenia elongata* (Spatangoidea), en el Golfo de Suez. Ambas son especies tropicales ampliamente distribuídas, mientras que el medio ambiente del Golfo de Suez es relativamente templado y tiene grandes fluctuaciones estacionales en la temperatura del mar.

La gametogénesis que lleva al desove empieza sincrónicamente en abril entre los individuos de *P. baculosa*, y el desove tiene lugar en julio y agosto. La actividad gametogenética está menos sincronizada entre los individuos de *L. elongata*, empieza a mediados y finales del invierno y el desove aparentemente tiene lugar continuamente durante la primavera y el verano. En ambas especies los fagocitos nutritivos están íntimamente relacionados con la gametogénesis. Estos fagocitos destruyen los restos de gametos y células gametogénicas que se forman fuera de estación y alimentan las células

gametogénicas que se forman durante el período reproductivo. Siendo la relación entre estas dos especies tan distante, el proceso general de gametogénesis descrito para ambas probablemente es igual en todos los equinoideos. Sin embargo, la falta en ambas especies de un largo período de almacenamiento de huevos es poco usual.

Las variaciones en temperatura, fotoperíodo y salinidad del mar no parecen estar directamente relacionadas con la sincronización de las periodicidades reproductivas de estas dos especies. Se sugiere, sin embargo, que la acumulación de nutrientes en los fagocitos nutritivos regula la gametogénesis y que esta acumulación puede estar indirectamente relacionada con las temperaturas y fotoperíodos del mar.

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