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Annual and lunar reproductive rhythms of the sea urchin, *Diadema antillarum* (Philippi) in Bermuda

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In the tropical Atlantic echinoid, *Diadema antillarum* in Bermuda, the gonads grow mainly in spring when sea temperatures are increasing, and spawning occurs from early summer to early winter. Peak spawning appeared to be in early summer and late fall. Gametogenesis is closely synchronized among different individuals and there is a well-defined lunar rhythm. Oocytes grow mainly between the first and third lunar quarters, and the animals spawn near the time of the new moon. Lunar synchrony, coupled with a spawning pheromone, may serve to maximize success of fertilization, while the restricted annual reproduction may maximize larval survival.

Bermuda; *Diadema*; lunar rhythms; reproduction; sea temperature; sea urchins

Introduction

Breeding periods of tropical echinoids of the genus *Diadema* have been described for different populations by a number of authors. Pearse [1] compiled this information and suggested that reproduction is limited to periods of higher sea temperatures in *D. setosum* of the tropical Indo-Pacific. Populations in northern (Japan, Suez) and southern (Australia) portions of the species' distribution spawn in the boreal and austral summers, respectively, when sea temperatures are above ~25°C. Recent studies in the Philippines, where sea temperatures are nearly always above ~25°C, revealed near continuous reproduction of *D. setosum* throughout the year [2], further supporting Pearse's suggestion. In contrast, reproduction of *D. antillarum* in the tropical Atlantic showed little pattern among different populations. Animals were reported to spawn throughout the year in Curaçao and the Virgin Islands [3], but mainly in the spring in Barbados [4], fall in the Florida Keys [5], and summer and early winter in Bermuda [6], with no clear relationship evident between reproduction and sea-temperature fluctuations.

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Besides annual reproductive rhythms, lunar rhythms are known for several species of sea urchins [7]. Particularly well-defined lunar gametogenic rhythms have been described for two species of diadematid sea urchins: *Diadema setosum* of the Indo-Pacific [8,9,10], and *Centrostephanus coronatus* in Southern California [11,12]. *D. antillarum* in the Florida Keys also has been reported to spawn between the new moon and the following full moon [5].

In this paper, we report the results of a 27-mth study on *Diadema antillarum* in Bermuda involving semimonthly measurements of gonadal indexes, and subsequent analyses of histological sections of the gonads. Bermuda, at 32°N, is the northern limit of the range of *D. antillarum* and experiences marked seasonal changes in sea temperature, ranging from about 17°C in January to nearly 30°C in August. The influence of temperature fluctuation on reproduction seemed evident in our samples. Moreover, our analyses demonstrated a well-defined lunar rhythm of gametogenesis and spawning.

Materials and methods

Specimens of *Diadema antillarum* were collected from the north side of the Causeway in Castle Harbour, Bermuda. This easily accessible site is the only inshore area where we found a large, natural population of these sea urchins. However, even though individuals of *D. antillarum* were abundant, there were not enough to sustain our regime of frequent sampling. Consequently, sections of the Causeway site were periodically restocked with animals collected from the fringing reefs of the South Shore of Bermuda where *D. antillarum* was most abundant, but where rough sea conditions often made sampling difficult. A comparison of the Causeway and South Shore populations from October–November 1980 revealed no significant difference in gonadal size, ripeness or histological condition between the populations (M.M. Blake, unpubl. data).

Four animals were collected semimonthly at the full and new moon from September 1978 to November 1980 and were dissected within a day. In July 1980, samples of 4 animals each were collected every 4 to 7 days. The animals were opened around the peristome, the lantern and gut removed, and the test inverted over a funnel on a graduated cylinder to collect eggs and sperms released through the gonopores. The gonadal index was calculated as the percentage of the test volume occupied by the gonads. Gonadal volume was measured by displacement, and test volume by using the formula for the volume of an ellipsoid on revolution about a minor axis: $V = 4/3\pi a^2 b$, where a is the radius of the ambitus and b the radius through the oral–aboral axis. The diameters were measured with calipers after the spines had been trimmed.

Whole gonads were fixed in 10% formalin for over 1 day. A portion of the oral tip of each gonad was embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin. Sections with abundant eggs and sperms were noted. In addition, size frequencies of the oocytes and ova in each ovary were analyzed as described by Pearse [13]. Sections of 50 oocytes showing a nucleolus, or ova showing a nucleus, were selected arbitrarily from each ovary and grouped into 4 size classes divided equally between 0 and 56 μm diameter. Live ova measured about 70 μm diameter while formalin-fixed ova and full-grown oocytes shrank to about 55 μm.

Results

Annual rhythm

The mean gonadal index fluctuated seasonally, increasing in spring as sea temperature increased, and decreasing in fall and early winter (Fig. 1). Abrupt decreases in the gonadal indexes suggest the beginning of spawning in spring with continuation into the winter. However, the seasonal pattern was much less evident in 1980 than in 1979. Moreover, relatively low mean values for samples taken in August and September of all 3 yr, followed by increases in October and November, suggests a bimodal pattern, with early summer and late fall being main spawning periods.

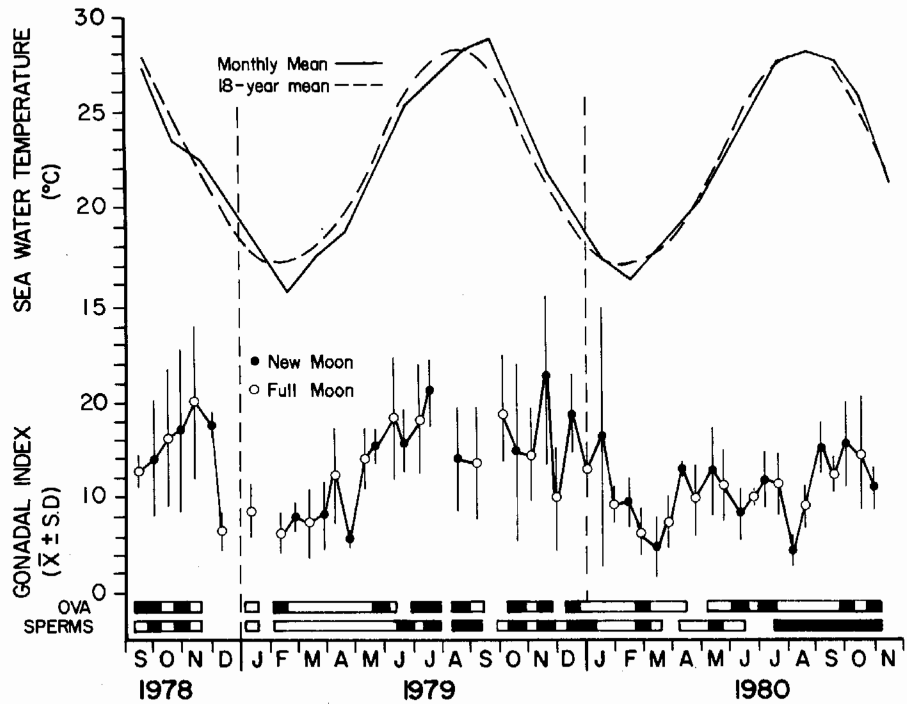

Fig. 1. Lower: gonadal indexes and presence (solid bars) or absence (open bars) of ova and numerous sperms in histological sections of the gonads; samples of 4 individuals each of *Diadema antillarum* collected in Bermuda on days of the new and full moon from September 1978 to November 1980. Upper: mean monthly sea temperatures from September 1978 to November 1980 and the 18-yr mean monthly sea temperatures taken at the Bermuda Biological Station on Ferry Reach, approximately 1.5 km from the collecting site on the Causeway.

Seasonal fluctuations in the presence of ova and sperms in histological sections corresponded with fluctuations of the mean gonadal indexes (Fig. 1). Gametes were found in few samples taken between January and May, while between May and December, they were present every month in samples taken at the new moon.

Taken together, the mean gonadal indexes and the presence of gametes in sections indicate an increase in reproductive activity in early spring, first spawning mid- or late spring, and continued gametogenesis and spawning to late fall or early winter. This long period of reproductive activity follows seasonal fluctuations of sea temperatures, with an increased activity occurring as sea temperatures increase above about 20°C and decreased activity following the decrease of sea temperatures below ~20°C. Moreover, the gonadal indexes were relatively low in mid-summer when sea temperatures exceeded 25°C.

Lunar rhythm

There was no statistical difference between mean gonadal indexes of the 73 animals collected at full moon (mean: 13.5) and the 76 animals collected at new moon (mean: 14.9) (t-test, $P < 0.2$), using the data collected only during the spawning season (April/May to December/January). However, 47% of the animals collected at the new moon during this time shed numerous gametes during dissection, while only 18% of those collected on the full moon did so. Using a t-test of paired monthly data, this difference between full and new moon samples was highly significant ($P < 0.01$). Histological analyses corroborated the observations made during dissections. Sections of gonads taken at the new moon between May and January contained ova or sperms, while few of the full moon samples contained gametes (Fig. 1). Moreover, in the oocyte-ovum size frequency analysis (Fig. 2), full-grown oocytes and ova were abundant in most samples taken at the new moon, but uncommon in most of the full moon samples, regardless of season.

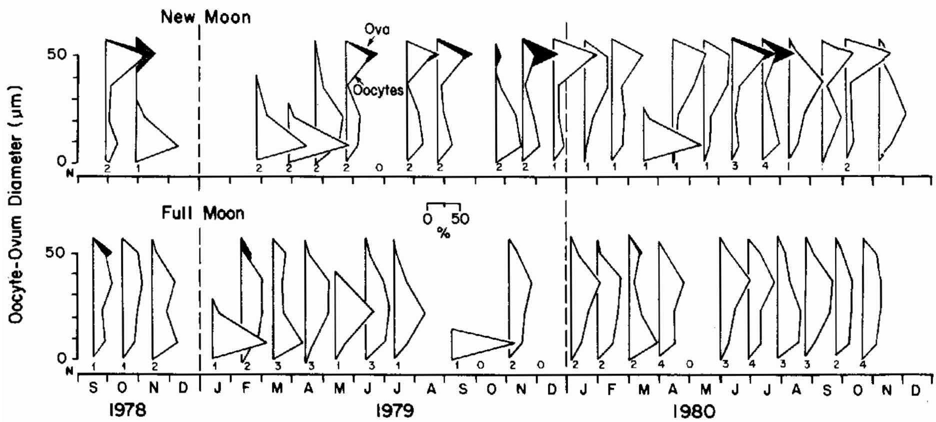

Fig. 2. Size distribution of oocytes and ova in females of *Diadema antillarum* collected in Bermuda on days of the new and full moon from September 1978 to November 1980. Each polygon represents the average for all females in each sample; n, number of females in each sample.

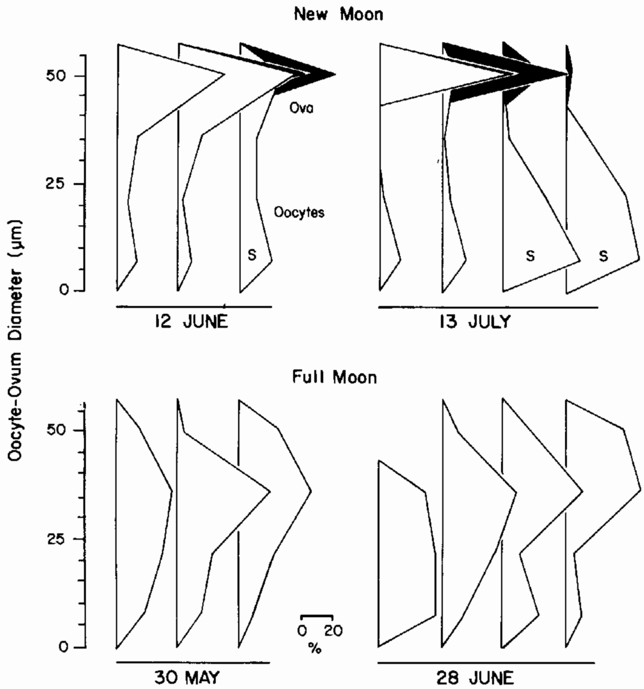

Fig. 3. Size distributions of oocytes and ova in all the individual females of *Diadema antillarum* collected in Bermuda on the days of the new and full moon of late May, June and mid-July 1980. 'S' indicates that the gonad contained few oocytes or ova, and that the animals had probably spawned recently.

The samples taken at the new and full moons of late May, June, and July 1980, all contained 3 or 4 females. Within each sample, the oocyte–ovum size frequencies were very similar among different animals, indicating close gametogenic synchrony (Fig. 3). Moreover, the animals taken on the same lunar phase in different months were in close gametogenic synchrony. Of the 7 animals taken at the new moon, 6 contained mainly full-grown oocytes or ova, and the seventh presumably had recently spawned most ova and contained small oocytes less than 25 μm in diameter. In contrast, 6 of the 7 animals taken at the full moon contained mainly growing oocytes, approximately 35 μm (formalin-fixed) in diameter, about 65% of the volume of full-grown oocytes.

Samples taken every 4–7 days in July 1980 revealed monthly changes in gonadal index, in oocyte size and in the number of individuals shedding numerous gametes during dissection ('ripe') (Fig. 4). Oocytes grew mainly between the first and third lunar quarters; by the end of this period, most of the oocytes were fully grown. Ova were most abundant at the new moon, and spawning occurred within the following 4 days. Oocyte growth was accompanied by an increase in gonadal index, and spawning by a sharp drop. The highest number of 'ripe' animals was recorded in the sample taken within days after spawning; the loose gametes were most conspicuous

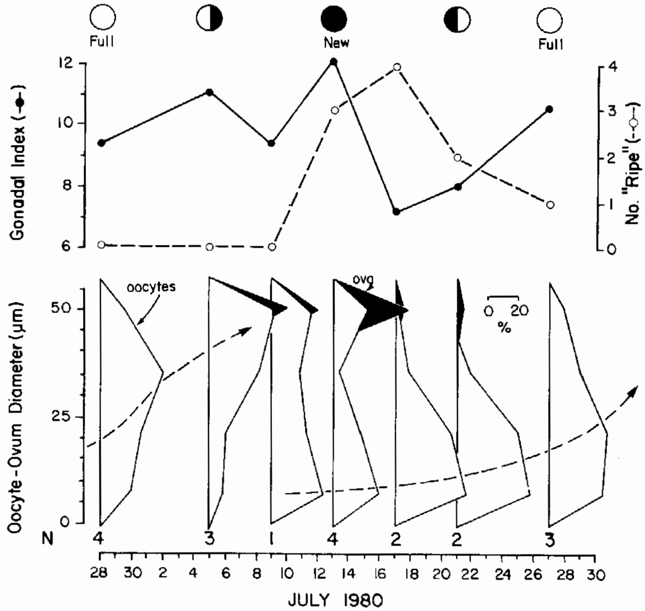

Fig. 4. Mean gonadal index, number of animals that shed numerous gametes on dissection ('ripe'), and size distribution of oocytes and ova in samples of *Diadema antillarum* collected in Bermuda over a lunar cycle in July 1980. Each polygon represents the average for all females in each sample; *n*, the number of females in each sample; dashed arrows indicate the suggested monthly course of oocyte growth.

then, even though they were relatively scarce and were likely relics that had failed to be spawned in the field.

Discussion

Annual rhythm

Our study confirms earlier reports that *Diadema antillarum* is ripe from late spring to early winter in Bermuda [6,14]. The annual cycle is indistinct, however, and probably variable among years. For example, gonadal sizes in summer–fall 1980 were considerably less than those reached in 1978 and 1979. Nevertheless, in both 1979 and 1980, the gonads increased in size in spring as temperature increased above 20°C; gonadal size then fluctuated erratically until the final drop in late fall or early winter after sea temperatures dropped below about 20°C.

Yonge [15] proposed that different species of tropical marine invertebrates could be categorized according to lower and/or upper sea temperatures that might limit their reproductive activity. He included *Diadema setosum* of the Indo-Pacific among these species that reproduce whenever sea temperatures are above ~25°C, and this inclusion has been supported by later work [1,2]. On the other hand, studies on *D.*

antillarum, including our own reported here, indicate that it should be placed in Yonge's category of species that reproduce between lower and upper sea temperatures of about 23 and 28°C. In Curaçao and the Virgin Islands, *D. antillarum* apparently is ripe throughout the year, but there is peak activity in winter and early spring [3]. Similarly, Lessios [16] recently reported that *D. antillarum* reproduces with little or no seasonality in Panama, where mean sea temperatures range between 26 and 28°C [17]. Reproduction is more restricted in Barbados where spawning occurs mainly in winter and spring when sea temperatures are near their annual minimum of about 26°C [4]. Similarly, gonadal growth and spawning occurs mainly in late fall and winter on the Florida Keys [5], when sea temperatures are below ~ 25°C (annual range ~ 20–30°C) [18]. Although our study indicates that reproduction of *D. antillarum* is restricted to periods when sea temperatures are above ~ 20°C in Bermuda, the relatively low gonadal indexes found in August and September, when sea temperatures exceed about 25°C, suggests that higher sea temperatures may inhibit gonadal growth as well.

Environmental factors other than sea temperature also could act as proximal cues that synchronize and regulate seasonal reproductive patterns in *D. antillarum*. Indeed, there is no compelling evidence that changing sea temperatures regulate reproductive rhythms in any species of sea urchin [19], despite suggestive correlations. Annual reproductive rhythms in *D. setosum* in several different parts of the tropical Indo-Pacific correlate particularly well with sea temperature changes, and spawning seems restricted to periods when temperatures exceed 25°C [1,15]. Nevertheless, experimentally increasing aquarium water temperatures to above 25°C in mid-winter had no effect on gametogenesis in animals from the Gulf of Suez [20]. Other environmental factors that may be more important as proximal cues regulating reproduction in tropical sea urchins include photoperiod, which regulates reproduction in the sea star *Pisaster ochraceus* [21] and the sea urchin *Strongylocentrotus purpuratus* (J.S. Pearse, V.B. Pearse and K.K. Davis, unpubl. data), and perhaps storms and food [19], or biotic factors such as population densities [16].

Diadema antillarum has a planktotrophic larva that spends weeks or longer in the plankton [15]. The ultimate cause of annual reproductive rhythms in species with planktotrophic larvae may be the maximizing of larval survival by timing larval production to periods either when larvae are least likely to be swept offshore or when phytoplankton production is the highest. Surface waters over the Bermuda Platform are generally conserved in summer [22] but constantly replaced in winter [23]. Many feeble swimmers are present in the plankton only in summer [24], and animals such as *D. antillarum* might conserve larvae by spawning mainly in early summer. On the other hand, primary production is highest in fall in Bermuda inshore waters [24] and in winter and spring in the surrounding Sargasso Sea [25]. Consequently, if food is critical, larval survival might be highest when spawning occurs in the fall. Larvae developing from gametes spawned in the fall, however, seem much less likely to return to Bermuda. Selection, if it is acting on the larvae of these animals, would also act on a more extended population, including animals in the Caribbean and Florida which spawn mainly in the fall and winter [3,4,5] and contribute larvae to the Gulf Stream and the Sargasso Sea Gyre.

Other common species of echinoids at Bermuda have spawning seasons similar to that of *Diadema antillarum.* The spawning period is spring and summer for *Lytechinus variegatus* ([18], D. McClay and R. Fink, pers. comm.), summer and fall for *Echinometra lacunter* [6], and early summer to early winter for *Tripneustes ventricosus* [6]. All these species, like *D. antillarum,* have long-lived planktotrophic larvae, and their continued presence in Bermuda may be due, at least in part, to spawning at times when the larvae are least likely to be swept away from the Bermuda Platform. Recent work on *L. variegatus* indicates that the population in Bermuda is distinct from those in Florida [26], and this population, at least, must be retaining larvae in Bermuda waters. On the other hand, all of these echinoid species are widespread and common in shallow waters of the tropical Atlantic, and they may spawn at times that maximize survival and successful dispersal in the Sargasso Sea Gyre.

Lunar rhythm

The lunar reproductive rhythm of *Diadema antillarum* in Bermuda appears similar to that reported for populations on the Florida Keys where Bauer [5] found smaller gonads nearer the time of the full moon than the adjacent new moons. However, we found no significant differences in gonadal sizes between full moon and new moon samples. Rather, we detected the lunar rhythm by observing the number of animals with numerous gametes in each sample and by histological analyses. The gonads of most echinoids are filled with both gametes and nutritive phagocytes [27], and differences in gonadal sizes do not necessarily indicate changes in gametogenesis or spawning. Consequently, detecting reproductive rhythms in echinoids by measuring gonadal sizes alone is difficult and often inaccurate.

Randall et al. [3] did not detect lunar reproductive rhythms in *Diadema antillarum* in the Virgin Islands and other sites in the West Indies from field observations of natural spawning or of spawning stimulated by lightly pushing on the test. Nevertheless, upon re-examining their data, we found that 9 of their 12 observations of spawning occurred within 5 days after the new moon and one additional animal spawned 8 days after the new moon. This is a highly significant deviation from random spawning (χ^2-test, $P < 0.001$). Thus, studies by Randall et al. [3], Bauer [5], and ourselves all indicate that *D. antillarum* spawns mainly near the time of the new moon, and that widely separated populations are closely synchronized with each other.

Lunar reproductive rhythms also have been described for *Diadema setosum* in Suez [8], Japan [9,10], and the Philippines [2] and for another diadematid, *Centrostephanus coronatus* in southern California [11,12]. Although spawning in these populations occurs shortly after the time of the full moon, other populations of both species apparently spawn at times of other lunar phases [11,20,28], suggesting that the timing is set differently in different populations [7]. To date there is little information on the proximal cues used by these animals to synchronize their lunar reproductive rhythms, but lunar rhythms of moonlight, rather than of tides, seem to be most important for maintaining the rhythm in *C. coronatus* on Catalina Island, southern California [12]. In some other species of marine animals, lunar reproductive rhythms are cued by different environmental signals in different localities [29], and the lunar as well as annual reproductive rhythms we found in *D. antillarum* may be regulated by several different factors.

Close reproductive synchrony expressed as a lunar rhythm may be mainly an adaptation maximizing fertilization, particularly for subtidal animals such as sea urchins which broadcast their gametes. Kennedy and Pearse [12] suggested that pheromones that stimulate spawning among neighboring conspecifics might be especially effective when the animals are brought into close gametogenic synchrony with a lunar rhythm. Randall et al. [3] observed epidemic spawning of *Diadema antillarum* in the field, and a pheromone that stimulates spawning of ripe conspecifics recently has been found in crude *D. antillarum* extracts and partially purified chromatographic fractions (T.M. Iliffe and J.S. Kittredge, unpubl. data). How this pheromone might be related to the lunar rhythm we describe awaits further investigation.

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References

1 Pearse, J.S., Reproductive patterns of tropical reef animals: three species of sea urchins, Proc. 2nd Int. Coral Reef Symp., 1 (1974) 235–240.

2 Tuason, A.Y. and Gomez, E.D., The reproductive biology of *Tripneustes gratilla* Linnaeus (Echinoidea: Echinodermata) with some notes on *Diadema setosum* Leske. Proc. Int. Symp. Mar. Biogeogr. Evol. Southern Hemisphere, Auckland, New Zealand, 17–20 July 1978, 2 (1979) 707–716.

3 Randall, J.E., Schroeder, R.E. and Starck, W.A. II, Notes on the biology of the echinoid *Diadema antillarum*, Caribb. J. Sci., 4 (1964) 421–433.

4 Lewis, J.B., Growth and breeding in the tropical echinoid *Diadema antillarum* Philippi, Bull. Mar. Sci., 16 (1966) 151–158.

5 Bauer, J.C., Growth, aggregation and maturation in the echinoid, *Diadema antillarum*, Bull. Mar. Sci., 26 (1976) 273–277.

6 Harvey, E.B., Bermuda sea urchins and their eggs, Biol. Bull. (Woods Hole, Mass.) 93 (1947) 217–218.

7 Pearse, J.S., Lunar reproductive rhythms in sea urchins. A review, J. Interdiscipl. Cycle Res., 6 (1975) 47–52.

8 Fox, H.M., Lunar periodicity in reproduction, Proc. R. Soc., London, 95B (1923) 523–550.

9 Yoshida, M., Some observations on the maturation of the sea-urchin *Diadema setosum*, Annotnes. Zool. Jpn, 25 (1952) 265–291.

10 Kobayashi, N. and Nakamura, K., Spawning periodicity of sea urchins at Seto. II. *Diadema setosum*, Publ. Seto Mar. Biol. Lab., 15 (1967) 173–184.

11 Pearse, J.S., A monthly reproductive rhythm in the diadematid sea urchin *Centrostephanus coronatus* Verrill, J. Exp. Mar. Biol. Ecol., 8 (1972) 167–186.
12 Kennedy, B. and Pearse, J.S., Lunar synchronization of the monthly reproductive rhythm in the sea urchin *Centrostephanus coronatus* Verrill, J. Exp. Mar. Biol. Ecol., 17 (1975) 323–331.
13 Pearse, J.S., Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. I. The echinoids *Prionocidaris baculosa* (Lamarck) and *Lovenia elongata* (Gray), Bull. Mar. Sci., 19 (1969) 323–350.
14 Mortensen, T., Contributions to the study of the development and larval forms of echinoderms I-II, K. Dan. Vidensk. Selsk. Skr. Naturvidensk. Math. Afd. 9 Raekke, 4 (1931) 1–39.
15 Yonge, C.M., The biology of reef-building corals, Gt. Barrier Reef Exped. 1928–29, Sci. Rep., 1 (1940) 353–389.
16 Lessios, H.A., Reproductive periodicity of the echinoids *Diadema* and *Echinometra* on the two coasts of Panama, J. Exp. Mar. Biol. Ecol., 50 (1981) 47–61.
17 Hendler, G., Reproductive periodicity of ophiuroids (Echinodermata: Ophiuroidea) on the Atlantic and Pacific coasts of Panama. In S.E. Stancyk (ed.), Reproductive Ecology of Marine Invertebrates, University of South Carolina Press, South Carolina, 1979, pp. 145–156.
18 Moore, H.B., Jutare, T., Bauer, J.C. and Jones, J.A., The biology of *Lytechinus variegatus,* Bull. Mar. Sci. Gulf Caribb., 13 (1963) 23–53.
19 Pearse, J.S., Synchronization of gametogenesis in the sea urchins *Strongylocentrotus purpuratus* and *S. franciscanus.* In W.H. Clark, Jr., and T.S. Adams (eds.), Advances in Invertebrate Reproduction, Elsevier/North-Holland, 1981, pp. 53–68.
20 Pearse, J.S., Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. III. The echinoid *Diadema setosum* (Leske), Bull. Mar. Sci., 20 (1970) 697–720.
21 Pearse, J.S. and Eernisse, D.J., Photoperiodic regulation of gonadal growth and gametogenesis in the sea star *Pisaster ochraceus,* Mar. Biol., 67 (1982) 121–125.
22 Boden, B.P., Natural conservation of insular plankton, Nature (London), 169 (1952) 697.
23 Boden, B.P. and Kampa, E.M., Winter cascading from an oceanic island and its biological implication, Nature (London), 171 (1953) 426.
24 Morris, B., Barnes, J., Brown, F. and Markham, J., The Bermuda Marine Environment, Bermuda Biol. Stn. Res. Spec. Publ., 15 (1977) 1–120.
25 Menzel, D.W. and Ryther, J.H., Annual variation in primary production of the Sargasso Sea off Bermuda, Deep-Sea Res., 7 (1961) 282–288.
26 Pawson, D.L. and Miller, J.E., Studies of genetically controlled phenotypic characters in laboratory-reared *Lytechinus variegatus* (Lamarck) from Bermuda and Florida (Echinodermata: Echinoidea). In J. Lawrence (ed.), Proc. Int. Echinoderms Conf., Tampa Bay, 14–17 Sept. 1981, A.A. Balkema, Rotterdam, in press.
27 Pearse, J.S., Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. II. The echinoid *Echinometra mathaei* (deBlainville), Bull. Mar. Sci., 19 (1969) 580–613.
28 Pearse, J.S., Patterns of reproductive periodicities in four species of Indo-Pacific echinoderms, Proc. Indian Acad. Sci., 678 (1968) 247–279.
29 Neumann, D., Tidal and lunar rhythms. In J. Aschoff (ed.), Handbook of Behavioral Neurobiology, Vol. 4, Plenum Publ. Corp., 1981, pp. 351–380.