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## Presence and absence of monthly reproductive rhythms among eight Caribbean echinoids off the coast of Panama

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**Abstract:** Monthly reproductive rhythms of five species of regular and two species of irregular echinoids were studied in the San Blas Archipelago during 1984. The annual reproductive periodicity of these species had been studied at the same locality in 1982–83. The data were subjected to Rayleigh's tests, autocorrelation analysis, and cross correlation analysis with the phases of the moon. Previously published data for an additional species, *Diadema antillarum*, that had been studied with the same sampling regime during the same time period in the same area, were re-analysed with the statistical procedures used in this paper. The eight species could be classified in four categories with regards to their mode of spawning: (1) *Tripneustes ventricosus* (Lamarck), *Echinometra viridis* A. Agassiz and *Leodia sexiesperforata* (Leske) spawn at random during the lunar cycle. (2) *Clypeaster rosaceus* (Linnaeus) and *Lytechinus williamsi* Chesher show nonrandom distributions of their spawning activity around the lunar cycle, but do not appear to follow any lunar periodicity. (3) *Euclidaris tribuloides* (Lamarck) and *Diadema antillarum* Philippi have a lunar rhythm in their reproduction, each spawning during a different lunar phase. (4) *Lytechinus variegatus* (Lamarck) follows a semilunar cycle, spawning every new and full moon, a pattern that had also been found in Bermuda in 1938. The phylogenetic position of the eight species indicates that lunar periodicity is not a lineage-specific trait, inherited from a common ancestor. It must, instead, have evolved independently in different echinoid taxa, possibly as an adaptation to serve the reproductive requirements of each species. However, attempts to identify its adaptive significance through comparisons between the species failed to support any of the hypotheses examined. Because water level on the Atlantic coast of Panama is determined by meteorological conditions as well as base tides, lunar spawning is unlikely to be cued by the tide, or to be an adaptation for flushing of fertilized zygotes away from reefs. Species in which lunar rhythms are present are not distinguished from species that spawn at random by their special photosensitivity, they are unlikely to be doing so because of food limitations during part of the month, and they do not appear to be under stronger selection for reproductive synchrony than species that have no lunar rhythms.

**Key words:** Lunar periodicity; Reproductive synchrony; Sand dollar; Sea urchin

### INTRODUCTION

Not long after Stevens (1936) wrote "One of the surest ways to incur ridicule among scientists is to suggest a relation between some natural phenomenon and the moon", it became apparent that lunar rhythms in the reproduction of many marine organisms was more than the subject of popular lore (Korringa, 1947). In echinoids, however, our knowledge of lunar reproductive periodicity remains very limited. When Pearse (1975)

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reviewed the existing literature on lunar reproductive rhythms of sea urchins, he could only treat two species, the diadematids *Diadema setosum* and *Centrostephanus coronatus*, and note that semilunar spawning had been shown in two additional species, *Lytechinus variegatus* (Moore et al., 1963) and *Mespilia globulus* (Kobayashi, 1967). Since then, another two diadematid species have been shown to follow a lunar cycle in their spawning, *Diadema antillarum* in the Caribbean (Bauer, 1976; Iliffe & Pearse, 1982; Lessios, 1984, 1988a) and *D. mexicanum* in the eastern Pacific (Lessios, 1984). The proximate and ultimate factors of such cycles remain obscure.

The first step in attempting to discern the reasons for the existence of monthly reproductive rhythms in echinoids is to establish which species follow a lunar reproductive cycle and which do not. This information can then help determine whether such cycles tend to be restricted to a few evolutionary lineages of echinoids, as their currently apparent limitation to the diadematids and temnopleuroids might suggest, or whether they might be independently derived adaptations. Obviously, to establish whether such patterns exist, we need to collect data for as many species in as many areas as possible and to report data not only from species that follow a lunar cycle but also from species that do not. It is not yet certain that "monthly reproductive rhythms are the exception among sea urchins", as Kennedy & Pearse (1975) have concluded, because presence or absence of reproductive periodicity has not been determined in most species. The value of looking for patterns of lunar reproduction in many species in the same areas is demonstrated in the study by Robertson et al. (1990), who were able to examine a number of hypotheses about its adaptive significance for fish by amassing data on which species reproduced with a lunar cycle, which species did so in a semilunar one, and which species showed no cycle at all. I am aware of only one study that has examined many echinoid species for the presence of lunar periodicity, that of Kobayashi (1969), who reported semilunar reproductive cycles in four out of ten sea urchin species in Seto. Unfortunately, sampling in his study was limited to only new and full moon, thus obscuring the distinction between semilunar cycles and continuous reproduction.

I studied the monthly reproductive rhythms of five species of regular echinoids, *Eucidaris tribuloides* (Lamarck), *Tripneustes ventricosus* (Lamarck), *Lytechinus variegatus* (Lamarck), *L. williamsi* Chesher and *Echinometra viridis* A. Agassiz, and of two irregular echinoids, *Clypeaster rosaceus* (Linnaeus) and *Leodia sexiesperforata* (Leske), in the San Blas Archipelago, off the Caribbean coast of Panama, during 1984. The annual reproductive periodicity of these species had been studied in 1982–83 (Lessios, 1985, in prep.). Data were collected in an attempt to answer the following questions: (1) Which of these species follow a lunar cycle in their spawning? (2) At what phase of the moon do species that spawn with a lunar cycle reproduce? (3) Is there a pattern in the mode of monthly spawning of these species that might suggest the reason for its existence and timing?

## MATERIALS AND METHODS

Each species was sampled during 1984 at 3-day intervals for 3 months during the active part of its annual reproductive season as determined from data gathered in 1982–83. Specimens of *E. tribuloides*, *L. williamsi* and *E. viridis* were collected at House Reef, in the vicinity of Punta San Blas (see Lessios et al., 1984a, for map). Specimens of *T. ventricosus* and *L. variegatus* were collected in *Thalassia* beds adjacent to this reef. *C. rosaceus* was gathered at the top of Aguadargana reef,  $\approx 500$  m east of House reef, and *L. sexiesperforata* at Tiantupu,  $\approx 500$  m southwest of House reef.

20 adult individuals of each species were collected at most sampling intervals, but in  $\approx 1\%$  of the cases sample size was as low as 17 individuals. The method of determining reproductive condition was identical to that used by Lessios (1984, 1985, 1988a). Each individual was placed in a separate container and injected with 0.5 M solution of KCl, which is known to induce spawning in ripe echinoids (Tyler, 1949; Hinegardner, 1975). *T. ventricosus*, *L. variegatus* and *C. rosaceus* received 10 ml KCl solution, the rest of the species 5 ml. The spawning response of each animal was classified in one of four categories, graded from no spawning to copious spawning, according to the volume of genital products they produced. The percent of animals spawning copiously was used as an index of the proportion of animals in the population that were ready to spawn on that day. Like histological examination of the gonads (Grant & Tyler, 1983a), or assessment of gonadal indices (Grant & Tyler, 1983b), this method measures readiness to spawn, rather than spawning activity. However, echinoids that are full of gametes usually release them readily in response to even slight mechanical stimuli; it is, therefore, likely that individuals that responded to KCl injections with copious gamete production would have spawned naturally within the next 3 days if they had been left undisturbed.

The same species had been sampled for 1 lunar month in 1983 under the same regime as 1984 (3-day intervals,  $20 \text{ ind} \cdot \text{species}^{-1} \cdot \text{time interval}^{-1}$ ), but the spawning response was classified in only two categories, i.e., spawning and no spawning. These data proved to lack the resolution necessary for determining reproductive periodicity, but they are presented here, because in some cases they provide indications as to whether rhythms suggested by the 1984 data are repeatable from one year to the next.

To determine whether distribution of spawning activity round the lunar cycle differed from random, Rayleigh's test (Batschelet, 1981, p. 54) was used. The lunar cycle was divided into arcs each corresponding to a 3-day sampling interval, and the number of animals spawning copiously during each interval was used to calculate a mean vector and Rayleigh's test, after correction for grouping (Batschelet, 1981, p. 37). If the pooled data from 3 months produced a significant value for Rayleigh's test, indicating nonrandom distribution of spawning during the lunar month, a mean vector was calculated separately for each lunar cycle to determine the phase of the moon in which spawning was concentrated. Each of these mean vectors was also used in a Rayleigh's test, limited to data from a single lunar cycle. Data from species that appeared to spawn at random were subjected to a second Rayleigh's test with the angles doubled to determine whether

they might spawn with a semilunar rhythm (Batschelet, 1981, p. 21). To determine the period of cyclicity, data from species in which Rayleigh's test produced significant results were analysed for autocorrelation, using the time series procedure T2 of BMDP (Liu, 1988). To determine the lunar phase of peak reproductive activity, the same data were entered into cross correlation analysis with the lunar cycle, represented by a sine wave with a 29.5-day period, truncated so that positive values cycled from 0 to 1, but negative values remained at 0.

A standard was needed to establish whether the sampling, assaying, and statistical techniques were capable of detecting lunar periodicity where it existed, and with which to compare patterns of reproduction of the species included in this study. To provide this standard, data for *D. antillarum* from Lessios (1988a) were re-analysed with the statistics applied to the other species. Inspection of the raw data (Bauer, 1976; Iliffe & Pearse, 1982; Lessios, 1984, 1988a) clearly indicates that reproduction in *D. antillarum* is concentrated in the first lunar quarter. The data from Lessios (1988a) were also collected in the San Blas in 1984 with the same techniques applied to the other species. The only difference is that in *D. antillarum* 2, rather than 3, months of data had been collected, which should make statistical significance in this species harder to demonstrate.

## RESULTS

The statistical tests used in this paper, when applied to *D. antillarum* (Fig. 1), produce results that lead to the same conclusions as those reached previously from simple plots of the raw data (Lessios, 1988a). Rayleigh's tests for all data and for each lunar cycle separately are significant, indicating nonrandom distribution of spawning activity. The mean vector of each cycle falls within the first 3 days after new moon. The meaningful lag (i.e., a lag larger than one sampling interval) with the highest autocorrelation coefficient corresponds to a period of 10 3-day sampling intervals, very close to what would be expected from a perfect lunar cycle of 29.5 days (9.8 sampling intervals). However, because the data span only two cycles, and because the period of the lunar cycle is not divisible by the sampling interval, the autocorrelation coefficient is not significant, an important deficiency in this study with regards to data from all species. The highest cross correlation coefficient with the lunar cycle is significant and occurs when the sine-wave that represents the lunar cycle is lagged by two 3-day intervals relative to the spawning cycle. As the sine-wave peaks at the end of the first lunar quarter, in the third sampling interval of each cycle, the cross correlations indicate that peak spawning occurs within 3 days after new moon, which agrees with the estimate provided by the mean vector of each cycle. Thus, the methods employed in this study are adequate to detect cycles that are distinctly lunar, with the caveat that a sampling series of 2 months is not long enough for significant autocorrelations. How do the data from the other species compare to those of *D. antillarum*?

Distributions of spawning activity in three species, *T. ventricosus* (Fig. 2), *E. viridis* (Fig. 3) and *L. sexiesperforata* (Fig. 4), are not significantly different from random. When the angles are doubled, Rayleigh's test statistic remains nonsignificant, indicating

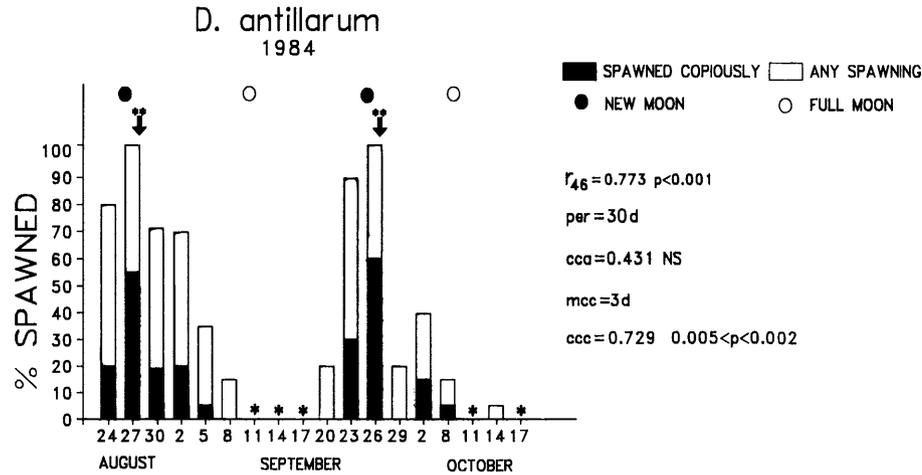


Fig. 1. Percent of *D. antillarum* spawning in response to 0.5 M KCl injections. Asterisks along x axis indicate days in which 20 individuals were injected but none spawned. Arrows in each lunar cycle indicate position of mean vector of timing of copious spawning. Asterisks over arrows indicate significance of a Rayleigh test limited to that cycle.  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously (significance after  $r_x$  comes from Rayleigh test from all data);  $per$ , period of spawning cycle (days) as suggested by lag with highest autocorrelation coefficient;  $cca$ , autocorrelation coefficient and significance;  $mcc$ , lag (days since new moon) that gives highest cross-correlation coefficient between spawning and lunar cycles;  $ccc$ , cross-correlation coefficient and significance; \*\*,  $0.005 < p < 0.01$ ; NS, not significant.

that spawning is random with respect to a semilunar cycle as well. *C. rosaceus* (Fig. 5) did not spawn during the 1st month of sampling in 1984, and spawned little during the 2nd month, indicating that the annual cycle suggested by 1983 data (Lessios, 1985) may not be consistent between years. There was copious spawning between 22 July and 18 August 1984. The concentration of spawning during the last month and the peak on 15 August, 4 days after full moon, has led to a significant overall Rayleigh's test. However, during July 1984, there was no copious spawning at all for 5 days after full moon. There is no significant cross-correlation between spawning and lunar phases, regardless of lag. Thus, this species shows a nonrandom distribution of spawning over one lunar cycle, but there is no evidence in the data for a consistent lunar rhythm. My previous claim (Lessios, 1987) that this species spawns with a lunar cycle was wrong. The 1st month of sampling of *L. williamsi* (Fig. 6) in 1984 showed a significant concentration of spawning in the first lunar half, but this trend was not repeated in subsequent months, nor is it reflected in the 1983 data. Doubling the angles indicates that no semilunar cycle exists either. The cross-correlation is not significant, and the period suggested by the

autocorrelation is not lunar. Thus, it is hard to imagine that this species follows the moon in its spawning, despite the significant results of Rayleigh's test.

In contrast to the preceding six species, in which there was at least a small amount

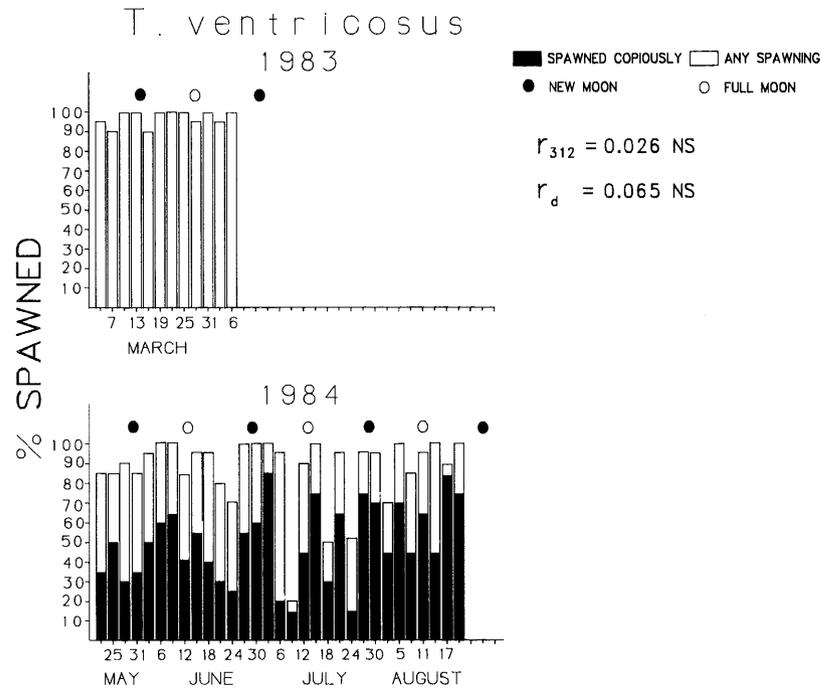


Fig. 2. Percent of *T. ventricosus* spawning in response to 0.5 M KCl injections.  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously;  $r_d$ , mean vector of data from all cycles with angles doubled (significance after  $r_x$  comes from Rayleigh test from all data); NS, not significant.

of genital products to be shed at any phase of the moon, *E. tribuloides*, like *D. antillarum*, contained no eggs or sperm during part of the month in both 1983 and 1984 (Fig. 7). Despite the small number of animals that responded to KCl injections, the results of Rayleigh's test are highly significant. The mean vector calculated from all 1984 data indicates that spawning is concentrated around 17 days after new moon, which agrees well with mean vectors calculated separately from each cycle, and with the interval of peak spawning suggested from the cross-correlation with the phases of the moon. With the exception of May 1984, the other months sampled during both years indicate a complete hiatus in reproduction around new moon. A slight shifting of the peaks between months, in part due to the fact sampling was done every 3 days, has caused the cycling period suggested by the (nonsignificant) autocorrelation to be shorter than

what would be expected from a true lunar cycle. Nonetheless, one can conclude that this species shows true signs of spawning regularly around full moon.

Spawning in *L. variegatus* (Fig. 8) shows no significance in Rayleigh's test when it is

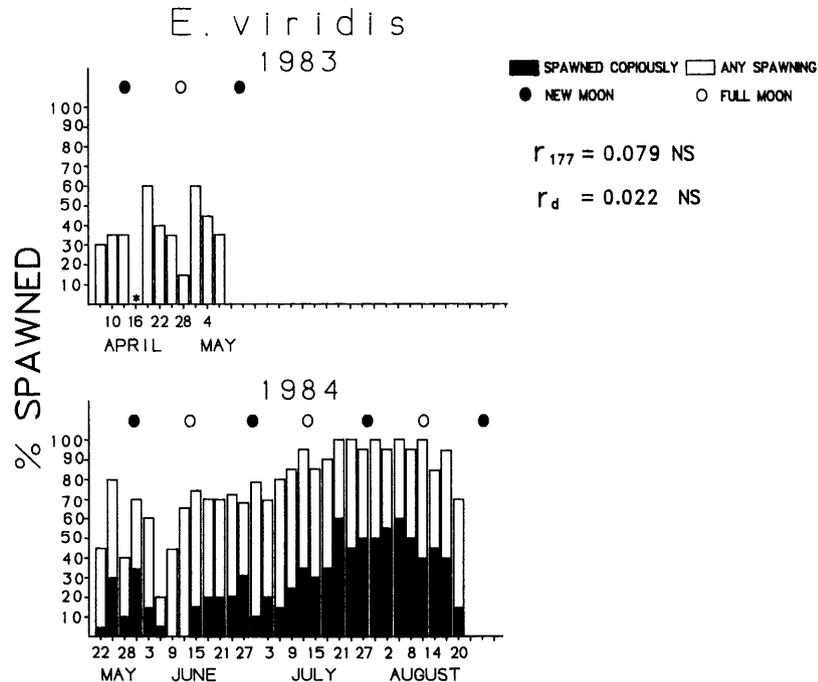


Fig. 3. Percent of *E. viridis* spawning in response to 0.5 M KCl injections. Asterisk along  $x$  axis indicates day in which 20 individuals were injected but none spawned.  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously;  $r_d$ , mean vector of data from all cycles with angles doubled (significance after  $r_x$  comes from Rayleigh test from all data); NS, not significant.

plotted in a lunar cycle, but does indicate significance when the angles relative to the origin (new moon) are doubled. This is an indication of a semilunar cycle, which agrees with the cycling period suggested by the (nonsignificant) autocorrelation. Accordingly, the percent of individuals that spawned copiously was cross-correlated with a sine-wave truncated so that it attains only positive values and cycling from new to full moon. In contrast to the cross-correlation with a lunar sine-wave (highest cross-correlation coefficient = 0.270, not significant) the cross-correlation with a semilunar sine-wave was significant, and indicated that peak spawning occurs immediately after new and after full moon, which agrees well with the mean vector calculated from all data (first sampling interval after new or full moon). Thus, the data suggest strongly that *L. variegatus* follows a semilunar cycle in its spawning.

## DISCUSSION

The eight species studied in the San Blas Archipelago fall in four categories with regards to their mode of spawning: (1) Three species, *T. ventricosus*, *E. viridis* and *L. sexiesperforata*, spawn at random during the lunar cycle. (2) Two species, *C. rosaceus*

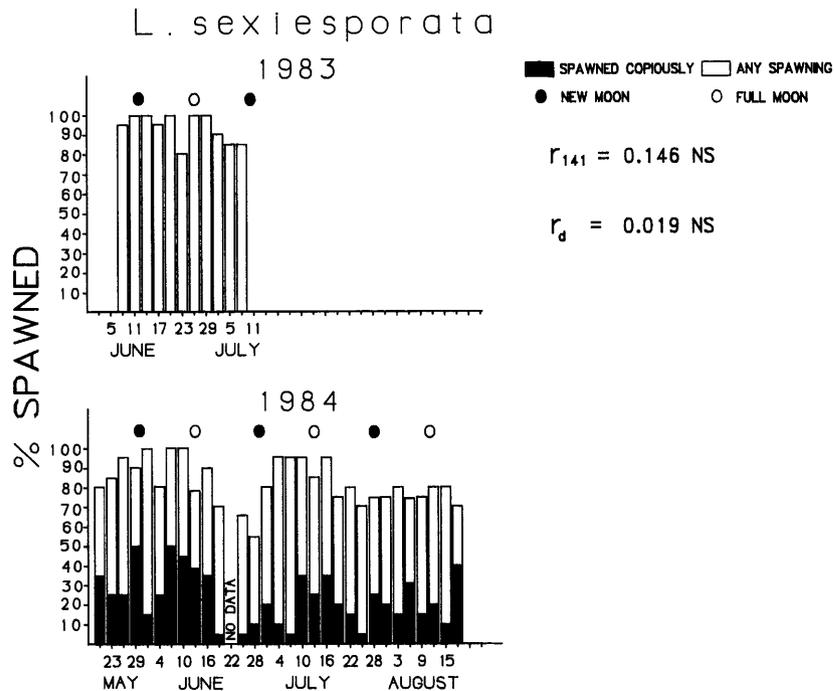


Fig. 4. Percent of *L. sexiesperforata* spawning in response to 0.5 M KCl injections.  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously;  $r_d$ , mean vector of data from all cycles with angles doubled (significance after  $r_x$  comes from Rayleigh test from all data); NS, not significant.

and *L. williamsi*, show nonrandom distributions of their spawning activity around the lunar cycle, but do not appear to follow any lunar periodicity. (3) Two species, *E. tribuloides* and *D. antillarum* have lunar rhythms in their reproduction, each spawning during a different lunar phase. (4) One species, *L. variegatus*, follows a semilunar cycle, spawning every new and full moon.

A question, that was first raised by Fox (1923), is whether lunar spawning in echinoids is cued by tides or moonlight. Pearse (1972) had favored the former, but Kennedy & Pearse (1975) produced evidence that *Centrostephanus coronatus* tracks lunar illumination rather than tides in its spawning cycles. That three species in the San Blas have reproductive rhythms that correspond with lunar cycles supports the view that

moonlight, rather than tides, may the *Zeitgeber* in monthly periodicity in echinoids. This is because water-level fluctuations in this area are slight, and do not necessarily follow the cycle of the moon; instead, they are chiefly influenced by meteorological conditions, which cannot be predicted for any given month (Glynn, 1972). *D. antillarum* is capable

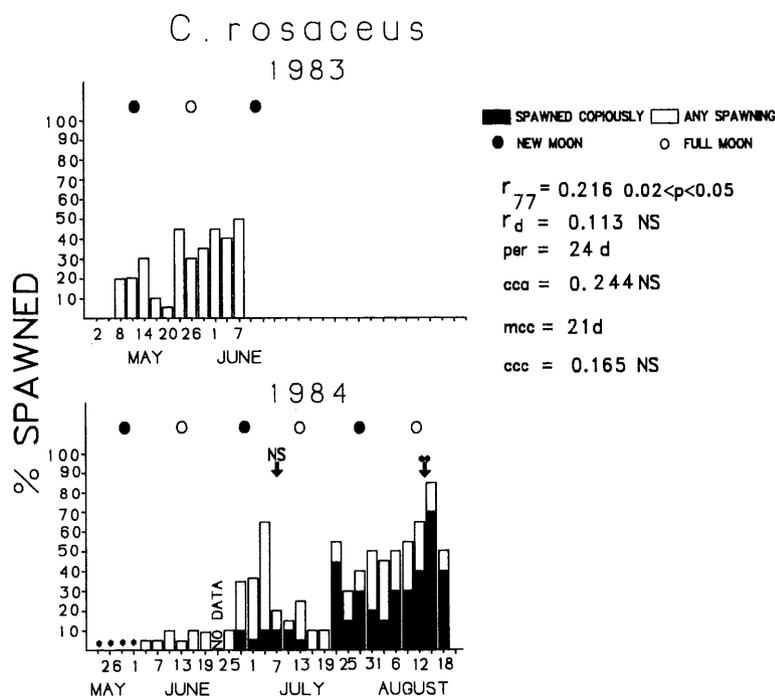


Fig. 5. Percent of *C. rosaceus* spawning in response to 0.5 M KCl injections. Asterisks along x axis indicate days in which 20 individuals were injected but none spawned. Arrows in each lunar cycle indicate position of mean vector of timing of copious spawning. Asterisks over arrows indicate significance of a Rayleigh test limited to that cycle.  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously;  $r_d$ , mean vector of data from all cycles with angles doubled (significance after  $r_x$  comes from Rayleigh test from all data);  $per$ , period of spawning cycle (days) as suggested by lag with highest autocorrelation coefficient;  $cca$ , autocorrelation coefficient and significance;  $mcc$ , lag (days since new moon) that gives highest cross-correlation coefficient between spawning and lunar cycles;  $ccc$ , cross-correlation coefficient and significance; \*\*,  $0.002 < p < 0.005$ ; NS, not significant.

of perceiving light, even in low intensities (Millott, 1954; Millott & Yoshida, 1959). Sensitivity to light has not been directly demonstrated in *E. tribuloides*, but its ability to shift its gametogenic cycles as the result of slight changes in photoperiod (McClintock & Watts, 1990), suggests that it can differentiate between light and dark and keep track of the time it spends under each regime. There is also some evidence of photosensitivity in *L. variegatus* (Millott, 1956; Sharp & Gray, 1962). However, photosensitivity would hardly explain why certain echinoids reproduce with a lunar pattern while others do not,

because it is a property of many species of echinoderms (Yoshida, 1966), including *T. ventricosus* (Lewis, 1958).

Part of the evidence that had led Pearse (1972) to suggest that echinoid reproduction may be cued to tides was that *Diadema setosum* spawns with a monthly rhythm

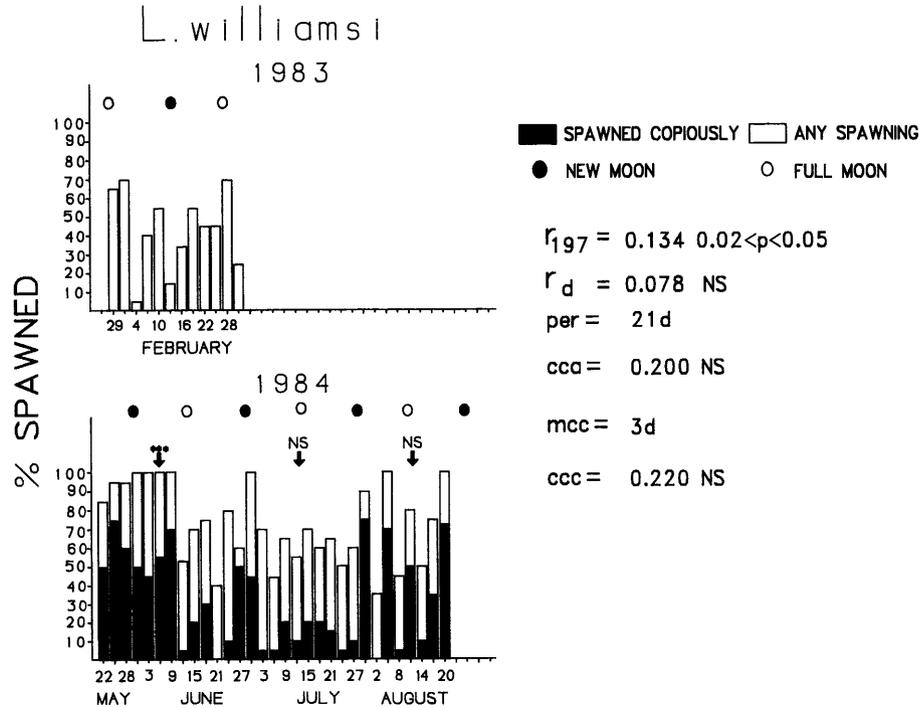


Fig. 6. Percent of *L. williamsi* spawning in response to 0.5 M KCl injections. Arrows in each lunar cycle indicate position of mean vector of timing of copious spawning. Asterisks over arrows indicate significance of a Rayleigh test limited to that cycle.  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously;  $r_d$ , mean vector of data from all cycles with angles doubled (significance after  $r_x$  comes from Rayleigh test from all data);  $per$ , period of spawning cycle (days) as suggested by lag with highest autocorrelation coefficient;  $cca$ , autocorrelation coefficient and significance;  $mcc$ , lag (days since new moon) that gives highest cross-correlation coefficient between spawning and lunar cycles;  $ccc$ , cross-correlation coefficient and significance; \*\*\*,  $p < 0.001$ ; NS, not significant.

everywhere, but the phasing of the cycles appears to be geographically variable (Pearse, 1968, 1970). As lunar phases are constant throughout the world but tides can vary locally, this implied that reproductive cycles might be tidal rather than lunar. However, *D. antillarum* spawns in the first lunar quarter in Bermuda, Florida, Panama, and the Virgin Islands (Ilfie & Pearse, 1982; Lessios, 1984; Levitan, 1988a). There are no published observations of spawning in *E. tribuloides* anywhere else in its range, but the

results obtained for *L. variegatus* at Panama in 1984 agree well with the results obtained at Bermuda by Moore et al. (1963) in 1938, both in regard to the existence of a semilunar cycle and in regard to the timing of the peaks. The agreement between mode

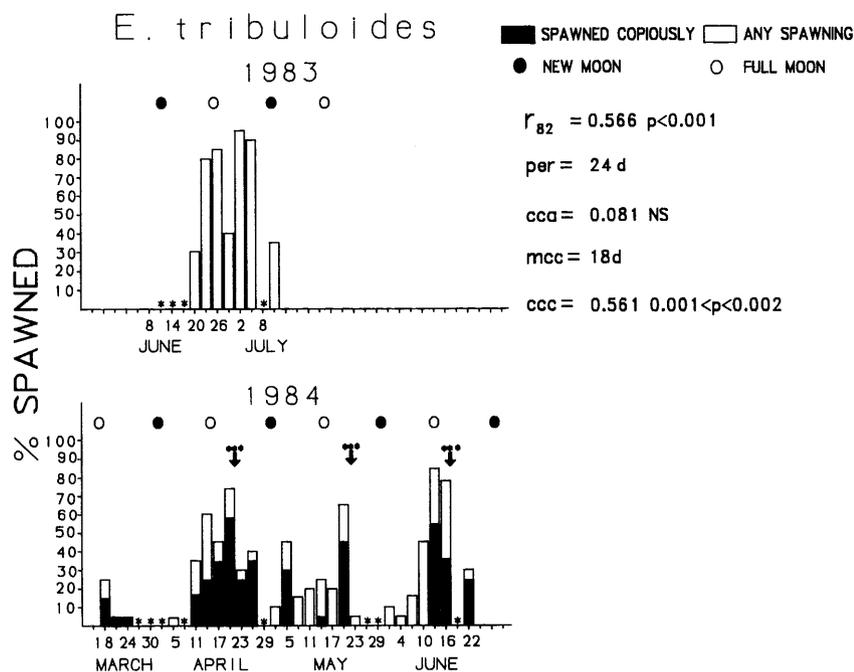


Fig. 7. Percent of *E. tribuloides* spawning in response to 0.5 M KCl injections. Asterisks along x axis indicate days in which 20 individuals were injected but none spawned. Arrows in each lunar cycle indicate position of mean vector of timing of copious spawning. Asterisks over arrows indicate significance of a Rayleigh test limited to that cycle;  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously (significance after  $r_x$  comes from Rayleigh test from all data);  $per$ , period of spawning cycle (days) as suggested by lag with highest autocorrelation coefficient;  $cca$ , autocorrelation coefficient and significance;  $mcc$ , lag (days since new moon) that gives highest cross-correlation coefficient between spawning and lunar cycles;  $ccc$ , cross-correlation coefficient and significance; \*\*\*,  $p < 0.001$ ; NS, not significant.

of spawning in such distant localities during different decades is remarkable in the presence of unusually high geographic variability in the morphology of this species (Serafy, 1973), variability that is genetically based (Pawson & Miller, 1982). Moore et al. (1963) were unable to demonstrate a significant correlation between lunar and spawning rhythms in *L. variegatus* females collected in the vicinity of Miami (though they found one in males), and suggested that lunar cycles may be present in Bermuda, because populations at the limit of the species distribution may be more sensitive to temperature fluctuations that may accompany tidal shifts. However, as Moore et al. (1963) sampled every 2 wk, an interval equal to the period of the cycle, it is likely that their Miami data

did not contain a sufficient number of points in different phases of the moon to produce a significant correlation. In view of the lunar cycles found in males at this locality, the conclusion that *L. variegatus* does not follow a semilunar cycle in Florida may have been premature. Whatever the case may be in Florida, the data from Panama indicate that semilunar cycles in *L. variegatus* are not restricted to marginal populations.

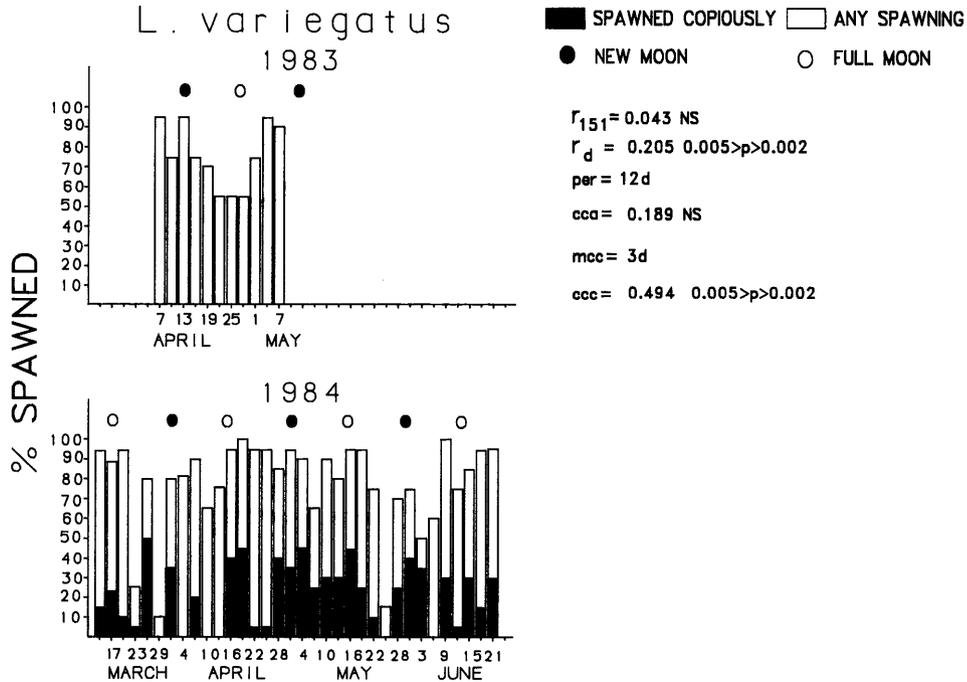


Fig. 8. Percent of *L. variegatus* spawning in response to 0.5 M KCl injections.  $r_x$ , mean vector of data from all cycles, where  $x$  is number of animals that spawned copiously;  $r_d$ , mean vector of data from all cycles with angles doubled (significance after  $r_x$  comes from Rayleigh test from all data);  $per$ , period of spawning cycle (days) as suggested by lag with highest autocorrelation coefficient;  $cca$ , autocorrelation coefficient and significance;  $mcc$ , lag (days since new or full moon) that gives highest cross-correlation coefficient between spawning and semilunar cycles;  $ccc$ , cross-correlation coefficient and significance; NS, not significant.

It is possible that a longer sampling series or a more refined sampling technique than those employed here might reveal departures from random spawning in the three species for which the present datum set could not reject this null hypothesis, or demonstrate cyclicity in the two species where spawning behavior was not correlated with the moon. However, the data from *D. antillarum*, *E. tribuloides* and *L. variegatus* indicate that a pronounced lunar or semilunar cycle can be detected with these techniques, even over a sampling series of two lunar cycles. Even if further sampling were to reveal some rhythmicity in *T. ventricosus*, *L. williamsi*, *E. viridis*, *C. rosaceus* or *L. sexiesperforata*, it

is doubtful that the rhythms would be sufficiently prominent to alter any conclusions based on the assumption that these five species are not lunar spawners. We, therefore, have the beginning of a quantification of how many echinoid species do and how many do not follow a lunar cycle in a given locality. That three out of eight species relate their reproductive cycles to the moon supports Kennedy & Pearse's (1975) assertion that the majority of echinoids do not have a lunar cycle in their spawning, but also suggests that lunar spawning may not be exceptionally rare in this class. Why do some echinoids reproduce with a lunar cycle, while others do not?

One explanation that always needs to be considered is that a set of species follow a particular life-history strategy, not because it is adaptive in their present ecological context, but because it was inherited from their common ancestor (Stearns, 1983, 1984; Clutton-Brock & Harvey, 1984; Harvey & Clutton-Brock, 1985; Lessios, 1990). To date, lunar cycles have been demonstrated convincingly only in the family Diadematidae (*D. antillarum*, *D. mexicanum*, *D. setosum* and *Centrostephanus coronatus*) and semilunar cycles in the order Temnopleuroida (*Mespilia globulus* and *L. variegatus*). Neither taxon has been reported to contain species that *do not* follow a lunar reproductive rhythm. Thus, the previously published evidence on lunar cycles in echinoids contained the suggestion that such cycles may be taxon specific. The data presented in the present paper indicate that this implied pattern was the result of insufficient sampling. *L. williamsi* does not emulate its congener in its mode of spawning; nor does *T. ventricosus*, another member of the Temnopleuroida show any lunar trend in its reproduction. Thus, it is unlikely that a lunar cycle in spawning is a life-history trait determined solely by phylogeny. That it has evolved independently in species that belong to three different orders suggests that it may be an adaptation to the reproductive requirements of the species that possess it. What adaptive explanations could be offered for the differences between the species in mode of spawning?

Hypotheses as to the adaptive significance of lunar spawning fall in two broad categories (Robertson et al., 1990): (1) "Offspring-survival hypotheses" maintain that certain phases of the lunar cycle are more favorable for the survival, dispersal, or settlement of the larvae; reproduction is timed to take advantage of these time periods. (2) "Adult biology hypotheses" allege that cycling of some environmental factor acting on adults makes reproduction possible only during certain parts of the lunar cycle. One can examine known aspects of the biology of each species in the hope that differences between species that follow a lunar cycle and those that do not might suggest the reasons for spawning with a monthly cycle.

One offspring-survival hypothesis that was proposed to explain lunar spawning for reef fish (Johannes, 1978; Ross, 1978; Robertson, 1983) is that spawning that coincides with spring tides during new or full moon assists in dispersal of fertilized eggs and larvae away from reef habitats and their associated planktivorous predators. It would be hard to see how this hypothesis could explain echinoid spawning in the San Blas, given the unpredictability of the tidal currents in this area. One can still argue that lunar spawners are trying to track the predictable component of tidal currents, i.e., the lunar base-tide,

or that their behavior in the San Blas may not be locally adaptive, but still follows a pattern that is advantageous in the largest part of the species range. However, a prediction of the tidal-flush hypothesis would be that, if the reef habitat is dangerous for newly fertilized eggs, lunar spawning would be more prevalent amongst reef-associated species. However, *L. variegatus* is not found on coral reefs, except as a sexually immature juvenile, yet it spawns in a semilunar pattern. Conversely, *L. williamsi* and *E. viridis* are restricted to coral reefs, but do not follow a lunar cycle. Other offspring-survival hypotheses have been proposed to explain lunar reproductive periodicity in marine organisms (e.g., Allen, 1972; Berry, 1986; Christy, 1986), but too little is known about larval biology and settlement patterns of echinoids to examine their applicability to this class.

Adult biology hypotheses invoked to explain lunar cycles in echinoids involve temperature (Moore et al., 1963; Kobayashi, 1969), and time available for feeding (Fox, 1967; Pearse, 1972). Moore et al. (1963) suggested that the Bermuda population of *L. variegatus*, being at the northernmost limit of the species, may be tracking fluctuations in temperature that may be associated with the tidal cycle. This explanation would certainly not apply to the San Blas populations of *L. variegatus*, *D. antillarum*, or *E. tribuloides*. The San Blas Islands, apart from having unpredictable tides, are located in the center of the species distributions, and can hardly be imagined to undergo lunar temperature fluctuations that dip below the minimum required for reproduction. Nor is it likely that a species like *L. variegatus*, which ranges well into the temperate zone, would be more limited in its temperature tolerance than the five tropical species that do not follow a lunar cycle.

Pearse (1972) examined the possibility that the lunar cycle might influence reproduction of *Centrostephanus coronatus* by affecting its rate of feeding during its nocturnal foraging. He rejected this hypothesis on the basis of observations of similar levels of night activity of this sea urchin regardless of lunar phase. An additional piece of indirect evidence against it is supplied by *D. antillarum*. An assumption of the hypothesis that increased feeding activity during new moon results in gonad production is that during other phases of the moon resource limitations prevent reproduction. Thus, this hypothesis would predict that a steep increase in the amount of food that can be gathered per unit time would result in a break-down of the lunar cycle, as the previously resource-limited animals become able to produce gonads at any phase of the moon. Population density of *D. antillarum* was reduced by >90% in Panama (Lessios et al., 1984a) and other places in the Caribbean in 1983 (Lessios, 1988b; Lessios et al., 1984b) due to mass mortality. This mortality resulted in unprecedented increases of food resources for the survivors and a concomitant elevation in their growth rate (Levitan, 1988b). However, the species continued to reproduce with a lunar cycle, even after resources became abundant (Lessios, 1988a; Levitan, 1988a). Thus food for the adults, unlikely to have been the cause of lunar cycles before the mass mortality, became even less credible as a factor after the majority of individuals died.

One hypothesis about the reasons of existence of lunar spawning cycles in echinoids

that seems eminently reasonable, but is very difficult to test, is that the main function of such cycles is to synchronize the reproduction of individuals in a population (Kennedy & Pearse, 1975; Iliffe & Pearse, 1982; Ims, 1990). For animals, such as echinoids, that broadcast gametes into the water column, that are limited in their ability to travel large distances in search of mates, and that need the proximity of freshly shed gametes for successful fertilization (Pennington, 1985), the need for reproductive synchrony is obvious. The hypothesis does not fit the adult vs. offspring dichotomy, because such synchrony might also enhance survivorship of fertilized zygotes and larvae by saturating potential predators.

If synchrony is the only impetus for reproductive periodicity, every species may follow its own cycle for no other reason than that a relative majority of the genotypes happened at some point in the population history to reproduce at a particular time, and other genotypes experienced selection pressures to join them. As a matter of fact, need for synchronous spawning does not necessarily imply need for periodic spawning; response to an environmental cue can synchronize all individuals in an area whether the cue occurs periodically or not. The only requirement is that the environmental trigger occurs often enough so that individuals full of gametes do not have to hold them for long periods of time, and rarely enough to allow the majority of individuals to manufacture new gametes after the last time that the stimulus was given.

The difficulty with testing the hypothesis that synchrony is the ultimate cause of reproductive periodicity comes from its ability to explain any nonrandom spawning pattern. The only obvious means of falsification is to generate predictions on the basis of the biology of each species as to the intensity of selection pressures it may have experienced for reproductive synchronization, and then see whether its spawning is periodic or not. Thus, Lessios (1985) examined the relationship between annual reproductive periodicity and population density in eight species of echinoids to see whether rare species tend to reproduce more rhythmically than common ones. The inverse relationship between the two parameters, expected if annual reproductive cycles were adaptations to achieve synchrony, was not found. Lunar cycles provide an additional mechanism of ensuring that conspecific individuals reproduce at the same time. There are also many factors in addition to population density that would affect the point density of gametes during spawning, and thus determine whether a species needs to reproduce synchronously. Point density of gametes would depend on test size of each reproducing adult and on the number of gametes that can fit in this cavity. Thus, small sea urchins with large eggs would be under stronger selection for reproductive synchrony than large sea urchins with small eggs. Point density of gametes would also be a function of the spatial distribution of adults during spawning, which in turn depends on the degree of their clumping under normal circumstances, plus their ability to move in order to form reproductive aggregations. That mobile sea urchins do not necessarily aggregate during spawning (Pennington, 1985; Levitan, 1988b), does not necessarily mean that mobility is unimportant. A species that is under selection to synchronize its reproductive cycle to counteract inadequate fertilization would also be under selection to form

spawning aggregations if it were not prevented from doing so by other factors. Passive clumping of adults is related to the type of habitat occupied by each species. Coral reefs tend to be patchy with respect to microhabitat preferred by each species; thus *L. williamsi* and *E. viridis*, species with high population densities on coral reefs, tend to be clumped in their distribution, whereas *L. variegatus* and *T. ventricosus*, occupying fairly uniform *Thalassia* beds, tend to be spread out.

How can these factors be combined in a meaningful test of the hypothesis that species that reproduce periodically do so because they need reproductive synchrony? Ideally, a composite index should be constructed to represent the potential point density of gametes in the water column for each species under the assumption that all individuals are ready to spawn as many gametes as they can produce. In the absence of information on nearest-neighbor distances and gonadal content at the time of spawning, one can examine each variable separately to form an impression of how important each of these factors may be.

Examination of Table I does not produce any obvious patterns for any one factor being of overwhelming importance in determining whether a given species will exhibit annual or lunar periodicity. The only species that has neither a lunar nor an annual cycle in reproduction is *T. ventricosus*, a large, fairly mobile sea urchin with small eggs, and with gonads that fill practically the entire body cavity during reproductive maturity (Lewis, 1958). However, population density of this species is low, and in the San Blas *Thalassia* beds individuals do not appear to clump during their daily activities. Lewis (1958) notes that in Barbados, where reproduction is seasonal, it tends to form aggregations during months of reproductive activity, and this might imply that individuals that are ready to spawn may seek each other out, thus mitigating the effects of low population density. *E. tribuloides*, the only species that shows both annual and lunar reproductive periodicity, has all the characteristics of a species under strong selection for reproductive synchrony. Its test diameter is small, its eggs are large, and it rarely, if ever, ventures out of its hole. Even though it inhabits coral reefs, its population density is so low, that clumping is of little help in bringing more than two or three individuals in proximity of each other. Thus, the two end-points of the continuum between periodic and random reproduction can be said to agree with the predictions of the hypothesis that the function of periodicity is to ensure synchrony. However, within this continuum, the results of an attempted correlation between what the hypothesis would expect and what is found are mixed. One would expect that mobility might counteract the effects of small population size in species that have an annual reproductive cycle, so that aggregations can be formed predictably, as they do in *Tripneustes* in Barbados. Table I indicates that, if one accepts this logic, species that do not spawn with monthly rhythms support the predictions of the synchrony hypothesis. Thus, the species with annual but no lunar cycles are either sedentary and abundant (*L. williamsi* and *E. viridis*), or rare but mobile (*C. rosaceus*). The degree to which *L. sexiesperforata* moves under the sand is not known, but its lack of lunar periodicity, despite its large eggs, can be explained as the result of its rather high population densities. It may even be of significance that

TABLE I

Distribution of spawning along lunar cycle, presence of annual and lunar periodicity, habitat type, tendency of adults to move, approximate adult test diameter, population density in 1983 and egg volume of each species on Atlantic coast of Panama. Data for annual periodicity and population density are from Lessios (1985) and data on egg size from Lessios (1990), unless otherwise noted. Adults were classified as mobile or sedentary on basis of field observations.

| Species                            | Annual periodicity | Lunar spawning pattern  | Habitat                                 | Adult mobility | Adult test diameter (cm) | Population density ( $N \cdot m^{-2}$ ) | Egg volume ( $\mu m^3$ ) |
|------------------------------------|--------------------|-------------------------|---|----------------|--------------------------|---|--------------------------|
| <i>E. tribuloides</i> <sup>1</sup> | Present            | Periodic                | Coral reef                              | Sedentary      | 2-3                      | 0.18                                    | $4.3 \times 10^5$        |
| <i>D. amillarum</i> <sup>2</sup>   | Absent             | Periodic                | Coral reef, sand, <i>Thalassia</i> beds | Mobile         | 4-9                      | 0.24                                    | $1.6 \times 10^5$        |
| <i>T. ventricosus</i>              | Absent             | Random                  | <i>Thalassia</i> beds                   | Mobile         | 8-12                     | 0.12                                    | $2.6 \times 10^5$        |
| <i>L. variegatus</i>               | Absent             | Periodic, bimodal       | <i>Thalassia</i> beds                   | Mobile         | 3-7                      | 0.12                                    | $5.2 \times 10^5$        |
| <i>L. williamsi</i>                | Present            | Nonrandom, non-periodic | Coral reef                              | Sedentary      | 2-3                      | 5.66                                    | $6.6 \times 10^5$        |
| <i>E. viridis</i>                  | Present            | Random                  | Coral reef                              | Sedentary      | 2-4                      | 48.77                                   | $4.0 \times 10^5$        |
| <i>C. rosaceus</i>                 | Present            | Nonrandom, nonperiodic  | Coral rubble, sand                      | Mobile         | 12-17                    | 0.08                                    | $1.7 \times 10^7$        |
| <i>L. sexiesperforata</i>          | Present            | Random                  | Sand                                    | ?              | 5-8                      | 1.84                                    | $6.7 \times 10^6$        |

<sup>1</sup> Data on annual periodicity from Lessios (in prep.) on population density from Lessios (1988a).

<sup>2</sup> Data on annual periodicity from Lessios (1981), on lunar periodicity from Lessios (1984) and Lessios (1988a), on population density after mass mortality from Lessios (1988b).

distribution of spawning through time in *E. viridis*, which outstrips all other species in density by an order of magnitude, cannot be distinguished from random, while *L. williamsi*, which lives a sedentary existence in the same habitat, has roughly the same size eggs enclosed in a body cavity of similar size, but is rarer than *E. viridis*, spawns synchronously, though not periodically. However, it is not clear why *L. variegatus* would need more than other species to reproduce in semimonthly synchrony. It is true that *L. variegatus* does not have an annual reproductive cycle in Panama, but this lack of cyclicity results from the fact that the majority of individuals are ready to spawn during any month of the year (Lessios, 1985). Its population density is low, but it tends to move, and its eggs are smaller and its body cavity much larger than those of *L. williamsi*. *D. antillarum* also fails to meet the predictions of the synchrony hypothesis. On the basis of its characteristics, this species would be expected to need reproductive synchrony as little as *Tripneustes*, because it is large and highly mobile. Its eggs are small, and its distribution tends to be clumped (Levitan, 1988a). In comparison to other species it is relatively abundant, even after the 1983 mass mortality. It does not have an annual reproductive cycle at Panama, but its lunar cycle is the best synchronized of all the species examined. Thus, two out of the eight species do not fit the predictions of the hypothesis that the main function of reproductive periodicity is to achieve reproductive synchrony. Given that with the present state of knowledge many patterns could have been explained by this hypothesis, the fact that it fails in 25% of the cases is sufficient to reject it as a general explanation of why some species reproduce with a lunar rhythm and others do not.

The evidence from the eight echinoid species from the San Blas Archipelago is almost entirely negative with respect to the attempted explanations applied to it: The species that spawn with a lunar rhythm are not likely to be influenced by the tide, nor do they seem to do so in order to flush their offspring away from reefs during spring tides; they are not distinguished from species that spawn at random by their special photosensitivity, they have not adopted this strategy because it is a characteristic of their lineage, they are unlikely to be doing so because of food limitations during part of the month, and they do not appear to be in greater need of reproductive synchrony than species that have no lunar rhythms. If each of these species had been studied separately, many of these factors might have appeared as reasonable explanations of the observed reproductive patterns. This alone should demonstrate the value of attempting to gather data on reproductive cycles from more than one species in an area.

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