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Asynchronous spawning and aggregative behavior in the sea urchin *Diadema antillarum* (Philippi)

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ABSTRACT: This study investigated spawning synchrony and aggregative behavior in Diadema antillarum over the lunar cycle. Urchins spawned on 25 days out of the 29 day lunar month, indicating that spawning is not highly synchronized. When urchins were seen to spawn spontaneously only 5% did so at one time. Slight aggregative behavior occurred throughout the entire lunar month, and did not change significantly over time. The data indicate that contrary to expectations, spawning is sporadic and occurs at low local density. This suggests that fertilization success is poor at the present time and may partially explain poor recruitment seen since the 1983 mass mortality of Diadema.

1 INTRODUCTION

The timing and distance of spawning individuals may have an important effect on the reproductive success of external spawners. Gametes have limited longevity and disperse quickly in sea water Pennington 1985). Periodicity in gametogenesis and spawning may increase reproductive success, since individuals are releasing gametes simultaneously. High population density and aggregative behavior may increase reproductive success, since individuals are releasing gametes in close proximity. Although Thorson 1946) suggested that benthic invertebrates typically spawn synchronously and in aggregations, spawning events have been infrequently observed Pennington 1985). Because of the paucity of data on spawning periodicity and local density of spawning echinoderms, it is difficult to estimate reproductive success in echinoderms.

Lunar reproductive periodicity is well documented for the sea urchin Diadema antillarum. Bauer 1976) investigated gonadal indices on the day of the new and full moon for 5 months and found larger gonads on the new moon. Iliffe and Pearse 1982) looked at both gonadal indices and size frequency distributions of ova and sperm on days of the new and full moon. Contrary to Bauer, they determined that there were no significant differences between gonadal indices between the new and full moon. However, they did find that oocytes and ova were significantly larger on the new moon. They also determined that significantly more animals shed gametes upon dissection at the new moon. Similar lunar patterns for other diadematid species have also been found reviewed in Pearse 1975).

The data on spawning synchrony and spawning densities are scarce. Bauer 1976) suggested that aggregative behavior is more pronounced in Diadema on the new moon. However, his data set was small 5 observations). Pennington 1985) sent out a questionnaire inquiring about observed urchin spawnings. Again the data set is small; he reports that 4 out of the 8 observed spawning events were in aggregated groups. Randall et al. 1964) reported that in 3 out of 13 sightings, small groups 4 - 10 individuals) of Diadema antillarum were seen spawning in close proximity.

The present study investigated lunar synchrony of spawning and aggregative behavior in the sea urchin Diadema antillarum. This study provides evidence that despite lunar periodicity and aggregative behavior, spawning events are typically asynchronous and spawning densities typically low.

2 METHODS

Patterns of spawning were monitored by direct observation during 4 years off the island of St. John, United States Virgin Islands. In addition, an experimental site was established in Lameshur Bay, St. John, USVI. At this site spawning readiness was assessed in a population of urchins by means of potassium chloride KCl) injection. Every 2 days for 5 months 25 urchins were selected from the population for KCl injection. Aggregative behavior was also quantified at this site using nearest neighbor analysis Vandermeer, 1981).

2.1 Direct observations of spawning

Direct observations of spawning were recorded during 4 years 1984 - 1987) of field work with Diadema. A total of 253 days were spent observing Diadema in the field. In addition, there were 25 night time observations. Spawning activity appears to be concentrated in the afternoon Lessios personal communication). Direct observations were made in two ways. Urchins were either seen spawning spontaneously or seen spawning after being picked up or probed by the author.

During the summer of 1987, spawning was seen during population census work Levitan, in prep.). When spawnings occurred spontaneously within established 5 x 5 m plots, the number of spawning and non-spawning individuals in this area was recorded. This provided information on the density of naturally spawning individuals and on the percentage of urchins that spawn simultaneously in a local area.

2.2 KCl induced spawning

Spawning following injection with KCl does not indicate that an urchin would have spawned naturally, on that day, but does provide a measure of the presence of mature gametes Thompson 1983) and an index of reproductive readiness. Since direct observations of spawning are often elusive, this may provide an adequate non-destructive procedure for examining spawning periodicity.

For this study, a 20 x 100 m monitoring site was established on the east side of Greater Lameshur Bay in 2 - 3 m of water. The site was subdivided into 20, 10 x 10 m areas. Every 2 days for 5 months starting Feb. 5, 1986), the 25 nearest urchins from a randomly selected area were collected. Once an area was selected it was removed from the sample pool for that lunar month. This reduced the chances of injecting the same urchin twice within the same lunar period. Collections were made between 1200 and 1800 hours. An urchin was placed in a boat and the test diameter recorded. The urchin was then injected with 0.5 ml of 0.5 M KCl. If this induced spawning, the sex was noted and the volume of released gametes measured to 0.01 ml. The urchin was then returned to the location from which it was collected.

Repeatedly injecting urchins with KCl, removed all mature gametes, however this method often resulted in mortality. For this reason only a single injection of KCl was used. A sample of 39 urchins was injected once and then repeatedly to estimate the relation between these methods. The results of this sampling procedure indicates that 0.5 ml injections provides a good relative estimate of total mature gametes female; gametes from single injection = 0.53 gametes from multiple injections + 0.20, $R^2 = 0.91$, $p < 0.0001$, male; single injection = 0.51 multiple + 0.24, $R^2 = 0.75$, $p < 0.0001$).

Volume measurements of spawn provide a relative estimate of gamete release. To insure that this measure remains constant over body size, a random sample of 25 egg volumes was examined microscopically with a hemocytometer. This established a mean value of 2.1×10^6 eggs/ml SE = 7.5×10^4). There was no significant linear relationship between body size and the density of eggs released F = 0.065, $p > 0.80$).

2.3 Patterns of Dispersion

Nearest neighbor distances were measured for all urchins selected for KCl injection. This provides a mean distance between urchins over the lunar period as well as allows a direct comparison of aggregation and reproductive readiness. In order to estimate the nearest neighbor distance indicating a random distribution, population density was estimated in 50 quadrats 5 x 5 m) along 4 transects parallel to the shore.

3 RESULTS

3.1 Direct observations of spawning

From 1984 to 1987, spawning activity was noted on 56 out of a total of 253 days spent observing Diadema in the field. On some days, as many as 30 urchins were seen to spawn. On no occasion were more than 3 to 4 urchins seen to spawn at the same

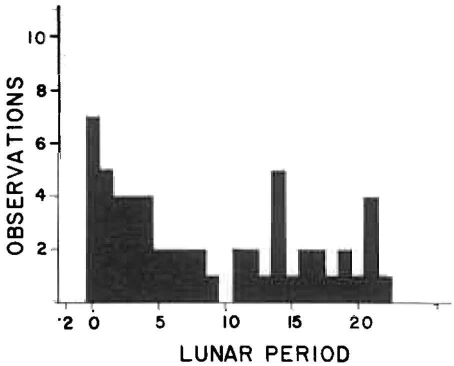

Figure 1. Diadema antillarum. Plot of incidental sightings of spawning over the lunar month. Day "0" indicates new moon. On some days several incidents of spawning were seen. Data were pooled as presence/absence for a single day. All observations made by author on the south side of St. John, USVI, between 1984 and 1987. There were 56 sightings out of 253 days of field observations.

Table 1. Spawning observations among undisturbed urchins. These seven observations were made during 1987 census work (Levitan in prep.), within 5 x 5 m quadrats. Lunar days are numbered with reference to the new moon (day 0); "No. spawning" are those urchins observed spawning within the 25 m^2 quadrat; "Dens. spawning" is the density of spawning urchins within the quadrat expressed on a m^2 basis; "No. urchin" is the total number of urchins in the quadrat; "Dens. urchin" is the total density of urchins in the quadrat expressed on a m^2 basis.

Lunar day	No. spawning	Dens. spawning	No. urchin	Dens. urchin
8	1	0.04	27	1.08
11	1	0.04	44	1.76
11	4	0.16	43	1.72
11	3	0.12	29	1.16
11	1	0.04	49	1.96
12	2	0.08	20	0.80
13	1	0.04	11	0.44
	1.86	0.07	31.86	1.27
	0.46	0.02	5.28	0.21

time in the same location 5-10 m^2 area). Data are reported as presence/absence for a single day and plotted over a 29 day lunar month (Fig. 1).

The distribution of spawning was significantly different from a uniform distribution of spawning over the lunar cycle (observed values pooled for each lunar quarter, Chi-square = 21.00, df = 3, $p < .001$). In contrast, the distribution of days spent observing in the field was not significantly different from a uniform distribution over lunar time (Chi-square = 11.12, df = 28, $p > .99$).

The period of most activity was the day of the new moon. There were 7 days of observation of spawning on the new moon. However, spawning was not limited to this time period. In fact, spawning occurred more than once on 16 out of the 29 lunar days (Fig. 1). It was only during the last 5 days of the lunar month that spawning was not observed. At no time was spawning noted during the night. Spawning was most common in the afternoon.

During 1987, within the 5 x 5 m quadrats, non-induced spawning was observed 7 times (Table 1). Observation time in each quadrat lasted 15 to 30 minutes. The mean density within the quadrats where spawning occurred was $1.27/m^2$ (SD = .56). However, an average of only 5.5% of the urchins present within the quadrat spawned simultaneously. This is equivalent to a mean density of

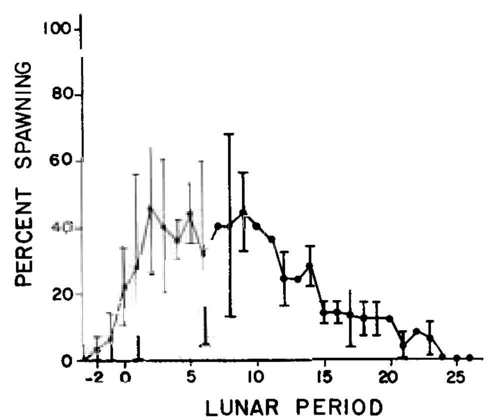

Figure 2. Diadema antillarum. Plot of percent spawning over the lunar month. Day "0" indicates new moon. All urchins injected from a 100 x 20 m site on the east end of Lameshur Bay, St. John, USVI. Every 2 days for 5 months (Feb.-Jun. 1986) 25 urchins were injected with 5 ml of .5 M KCl. The mean for each lunar day and standard deviation are plotted.

spawning urchins of only $0.07/m^2$ (SD = 0.05). There were no observations of spawning urchins within 30 cm of one another.

3.2 KCl induced spawning

The pattern of spawning seen in urchins injected with KCl was similar to that seen in the direct observations (Fig. 2). The number of urchins spawning after KCl injection was significantly correlated with lunar time (linear regression of number spawning over lunar time was significant $R^2 = .50$, $F = 60.2$, $p < 0.0001$, $n = 62$). Spawning in the injected urchins started 2 days sooner, and peaked 2 days after the direct observations of spawning. Peak spawning activity lasted 10 - 12 days (40 - 60 percent of the injected urchins spawning). Spawning activity continued through day 23 (10 - 20 percent spawning) and then stopped for the remaining 3 days.

There was no relation between the number of gametes released per spawning individual and the lunar period (linear regression of volume of gametes released vs lunar time not significant $F = 0.79$, $p > 0.37$, $n = 140$).

There was a significant relationship between body size and the number of gametes released when spawning (Fig. 3). For both male and female urchins, larger animals released more gametes than smaller animals. However, the relationship was quite variable.

There was no relation between body size and the frequency of spawning among urchins of reproductive size (25 mm test diameter and larger) (Table 2).

Male urchins were more likely to spawn when injected with KCl than female urchins (Table 2). The sex ratio of spawning urchins was 73% male. This value did not reflect the population sex ratio, which was 48% male, based on a sample of 39 dissected urchins.

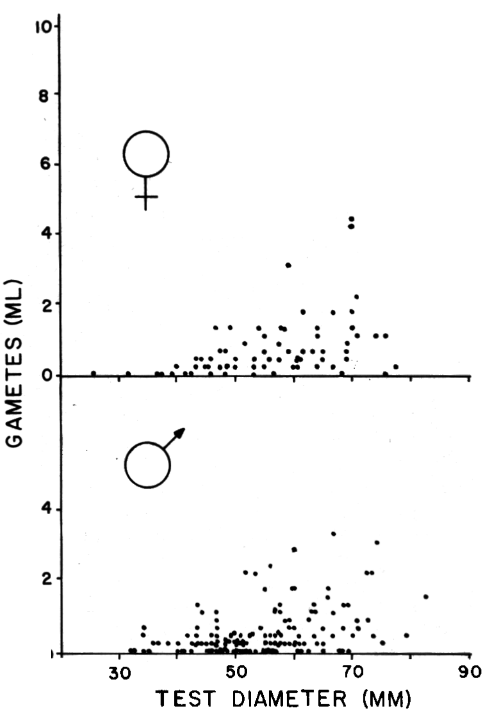

Figure 3. Diadema antillarum. Volume (ml) of gametes released when injected with 0.5 ml of 0.5 M KCl plotted against body size (mm test diameter). Only urchins that released gametes are plotted. A) Female urchins; log gametes = log size 3.56 - 6.59, $R^2 = .35$, $p < .001$, $n = 74$. B) Male urchins; log gametes = log size 2.30 - 4.58, $R^2 = .10$, $p < .001$, $n = 134$.

3.3 Patterns of dispersion

There was no apparent lunar pattern of aggregative behavior (Fig. 4). Urchins

Table 2. Number and percent spawning by size class (mm) when injected with 0.5 ml of 0.5 M KCl. Chi-square of number of urchins spawning versus size class = 14.82 NS, $p > .05$, df = 8 (not including 20-25 mm size class).

Size	Injected	Spawned	Male	Female
20-25	1	0	0	0
25-30	14	1	0	1
30-35	77	11	9	2
35-40	143	20	16	4
40-45	249	54	41	13
45-50	383	91	67	24
50-55	338	68	53	15
55-60	200	54	39	15
60-65	76	23	13	10
65-70	49	11	6	5
> 70	26	3	1	2
Total	1556	336	245	91

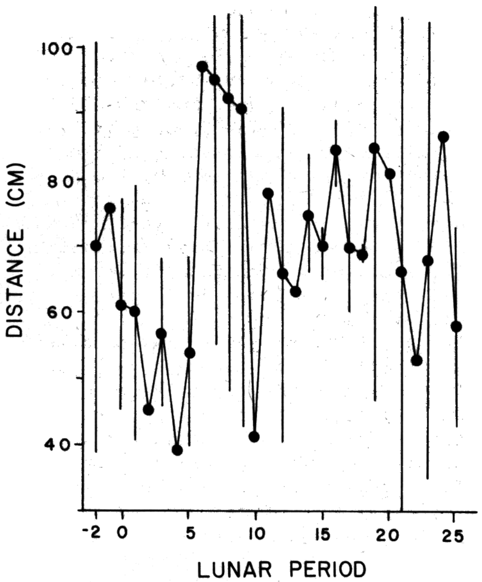

Figure 4. Diadema antillarum. Plot of nearest neighbor distances over the lunar month. Day "0" indicates new moon. Data collected at same time as Fig. 2. The mean for each lunar day and standard error are plotted. A random distribution would be a nearest neighbor distance of 132 cm. Density at this site was $0.14/m^2$.

had a clumped distribution that was independent of lunar time (linear regression of distance vs lunar time not significant, $F = 0.44$, $p > .50$, $n = 55$). The mean nearest neighbor distance was 70 cm (95% confidence interval = 6.4). The population density at this site was $0.14/m^2$. A distance of 132 cm would indicate random distribution.

There is also no relationship between reproductive readiness and aggregation. The mean nearest neighbor distance was 74.65 cm for spawning urchins and 74.98 cm for non-spawning urchins (Student's 't' = 0.04, $p > .9$, NS).

4 DISCUSSION

Lunar periodicity in gametogenesis is well established for Diadema antillarum (Bauer 1976, Iliffe and Pearse 1982). However, the presence of a lunar cycle reveals very little about reproductive synchrony. In order for periodicity to be of consequence in terms of reproductive success, urchins must shed their gametes simultaneously. The present study concurs that a lunar cycle is present; an urchin is more likely to spawn on the new versus the full moon. However, spawning activity is not limited to the time of the new moon: individuals spawned on 25 out of 29 lunar days. The KCl induced spawning data suggest that even at a single location spawning is scattered over the lunar month. This protracted spawning period may tend to decrease average fertilization success.

The data of Randall et al. (1964) supports this view that spawning is scattered over the lunar period. Their data (13 incidental sightings of spawning activity) indicate that spawning is most prevalent within the first quarter of the moon, but include observations as late as the 19th day of the cycle (a significance test of this data was conducted by Iliffe and Pearse 1982).

Aggregative behavior, even if it is independent of spawning, could tend to increase spawning success. The amount of clumping seen indicates that Diadema are closer together than would be expected by random (mean nearest neighbor distance of 70 cm versus 132 cm for random). However, the effect of aggregation may be offset by sporadic spawning. When urchins were seen to spawn spontaneously only 5% did so at one time. The importance of local density on fertilization success is still largely unknown, thus conclusions at this point must be tentative. Experimental work in this area has just begun (Pennington 1985).

Although a substantial amount of time was spent observing Diadema in the field, it is possible that major spawning events were missed. The chance of this occurring in the KCl induced spawning site are slight, since individuals slowly decreased from a peak reproductive readiness. This pattern indicates sporadic spawning. A peak of readiness, followed by a sharp decline would indicate a widespread spawning event. The pattern of peak spawning followed by a slow decline was seen in the direct observations. This provides evidence that natural spawning events are also sporadic. However, the number of gametes being released from these individuals is unknown. The possibility exists that sporadic spawning, of a relatively small number of gametes, might signal reproductive readiness. If other ready individuals are present, in enough numbers, this might lead to a major spawning event. Although this is possible, it was never observed.

Rokop's (1974) research on deep sea organisms indicated that synchrony appeared to be less common when organisms exhibit relatively high population density. Rokop suggested that in these cases the selective pressure to spawn simultaneously may be reduced because the probability of spawning at the same time as a nearby conspecific increases with population density. Historically, Diadema antillarum has been at high density (Bauer 1980, Sammarco 1980, Carpenter 1981). Perhaps when population density was high, selective pressure on synchrony was relaxed. Lessios (1981) investigated annual synchrony of gonadal indices in two populations of Diadema antillarum. His data indicated that synchrony was tighter at the site with lower population density.

Mean population density in Lameshur Bay was $14/m^2$ prior to 1983 (Levitan in prep). During 1983, a mass mortality of Diadema reduced population density 100 fold throughout the Caribbean (Lessios et al. 1984, Bak et al. 1984, Hughes et. al 1985, Hunte et al. 1986). Since the mortality event, recruitment in Lameshur Bay (Levitan, in prep.), and elsewhere in the Caribbean (Lessios, this volume), has been poor. This may be due to a reproductive strategy unsuitable for the present low density conditions. Diadema may be adapted for reproducing in populations of higher average density. Recovery of population density to former levels might therefore be expected to be slow.

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