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Temporal and spatial variation in egg size of 13 Panamanian echinoids

H. A. Lessios

Smithsonian Tropical Research Institute, Balboa, Panama

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Abstract: Though egg size of marine invertebrates has received a great deal of theoretical attention, and though sea urchin eggs have been studied for nearly a century, practically no data on intraspecific variation of echinoid egg size exist. This study examines temporal variation in egg size of 10 species of regular and three species of irregular echinoids from either side of the Isthmus of Panama. Data on spatial variation in egg volume are also presented for eight of these species. In all species mean size of eggs from different females collected from the same locality on the same day were significantly different. Daily (within-month) variation in egg size was significant in three out of the five species for which sufficiently large samples were collected: *Eucidaris tribuloides* (Lamarck), *Diadema antillarum* Philippi, and *Lytechinus variegatus* (Lamarck). No significant differences between daily means of egg volume were evident in *D. mexicanum* A. Agassiz and in *Echinometra viridis* A. Agassiz. Monthly means of egg volume were significantly different in seven out of the 13 species studied: *Lytechinus variegatus*, *L. williamsi* Chesher, *Tripneustes ventricosus* (Lamarck), *Echinometra viridis*, *E. vanbrunti* A. Agassiz, *Clypeaster rosaceus* (Linnaeus), and *C. subdepressus* (Gray). Between-year variation was also significant in all of these species, except *Lytechinus variegatus*. All Caribbean species with significant monthly variation show a decline in egg size after September, but no obvious environmental fluctuation can be linked to this change. *Echinometra lucunter* (Linnaeus) showed no significant variation between months of the same year, but exhibited differences between years. No significant monthly or annual variation was observed in *Eucidaris tribuloides*, *E. thourarsi* (Valenciennes), *Diadema antillarum*, *D. mexicanum*, or *Leodia sexiesperforata* (Leske). When temporal variation at each locality was taken into account, only one species out of eight, *Clypeaster subdepressus*, showed spatial variation. Correlations between size of eggs collected at a particular time and the intensity of spawning by the population at that time were generally not significant, suggesting that size of mature eggs is not determined by reproductive state of the parental population. Correlations were also attempted (in three species of *Echinometra*) between egg size and body weight, gonadal content, and number of eggs carried by the mother. None of these variables was a good predictor of egg size. Only in *E. viridis* was there a significant correlation between egg size and gonadal content. Though its causes remain obscure, intraspecific variation in egg size found in these echinoids could be exploited to study the possible effects of this parameter on larval life history.

Key words: Caribbean; Eastern Pacific; Echinoderm; Geographic; Intraspecific; Life history

INTRODUCTION

Egg size of marine invertebrates has been the object of many theoretical treatments because it is a life history parameter intricately related to mode of development and to

Correspondence address: H. A. Lessios, Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama.

allocation of resources (e.g., Vance, 1973; Christiansen & Fenchel, 1979; Perron & Carrier, 1981; Grant, 1983; Strathmann, 1985; Emler, 1986; Emler *et al.*, in press). In the search for adaptive significance of egg dimensions, inductive tests of theoretical predictions and deductive explorations for patterns have relied on interspecific, rather than intraspecific, comparisons (e.g., Thorson, 1950; Underwood, 1974; Spight, 1976; Strathmann, 1977; Strathmann & Vedder, 1977; Turner & Lawrence, 1979; Perron, 1981; Amy, 1983; DeFreese & Clark, 1983; Lawrence *et al.*, 1984; McEdward, 1986a,b; Emler *et al.*, in press). Interspecific comparisons, despite their obvious limitations (Vance, 1974; Grant, 1983; Felsenstein, 1985), have been necessary because of the paucity of data on intraspecific variation of marine invertebrate egg size. Within-species comparisons, however, may be much more useful in elucidating life history correlates of egg size, because confounding variables are kept to a minimum (Grant, 1983). In fishes, for example, studies of intraspecific egg size variation have revealed a number of patterns. Size of fish eggs has been found to vary with season (Bagenal, 1971; Southward & Demir, 1974; Ware, 1975, 1977; Daoulas & Economou, 1986), to be dependent on the size of the mother (Mann & Mills, 1985), and to affect the survivorship of the offspring (Blaxter & Hempel, 1963; Wallace & Aasjord, 1984). With few exceptions (e.g., Barnes & Barnes, 1965; McEdward & Carson, 1987; McEdward & Coulter, 1987), studies of temporal or spatial variation in egg size have not been carried out in marine invertebrates.

Echinoids, despite the attention they have received from embryologists, are no exception to the pattern of ignorance about intraspecific egg size variation (Emler *et al.*, in press). Though early articles noted variation in egg measurements (e.g., Glaser, 1924; Goldforb, 1935; Shapiro, 1935; Harvey, 1956) and even mentioned that egg size seemed to change with season (Goldforb, 1935), no systematic investigation of temporal variation of echinoid egg dimensions has been published. The most complete data come from a comparison of eggs from 16 individuals of *Strongylocentrotus droebachiensis* collected at two different times, 13 months apart; they showed no significant differences (Turner & Lawrence, 1979). Spatial variation in egg diameter was reported in *Psammochinus miliaris* by Lönning & Wennerberg (1963) and in *Strongylocentrotus pallidus* and *S. droebachiensis* by Hagström & Lönning (1967).

This paper examines temporal variation in egg size of 13 echinoid species from the two coasts of Panama. It asks whether eggs collected from different individuals on the same day, on different days of the month, in different months of the year or in different years vary in volume. Data from different localities are used (where available) to determine magnitude of geographic variation. An attempt is made to understand the biological basis of intraspecific variation of egg size through correlations with reproductive activity over time and with attributes of the individuals that contained the eggs.

MATERIALS AND METHODS

Eggs were obtained between 1976 and 1985 from 10 species of regular echinoids and three species of clypeasteroids. Ten of these species inhabit the Caribbean and three the eastern Pacific (Table I). In general, one or two localities per species were sampled once a month (on the same phase of the moon) for a minimum of a year to determine annual patterns of reproductive periodicity (Lessios, 1981, 1985, in prep.) and every three days for a minimum of a month to examine lunar periodicity in spawning (Lessios, 1984, in prep.). Approximately 20 animals per species per locality were sampled at each time interval. The specimens used in the study of reproductive periodicity also provided the eggs for the present study. Though sampling was done at regular intervals, eggs from sexually ripe females were not always available. In addition to the regular sampling associated with the study of reproductive periodicity, collections of eggs were made at other times and in other localities whenever ripe females of a particular species could be located (Table I).

Animals were induced to shed their eggs into bowls full of sea water by injection of 0.5 M solution of KCl in the coelomic cavity (Tyler, 1949). An exception to this general procedure was made in 1976–77 for the three species of *Echinometra*; eggs from these species during these years were obtained by dissection (see below). Unless otherwise specified, eggs were preserved in 5% formaldehyde solution in sea water within 0.5 h of their shedding and measured at a later time. Only measurements from ripe eggs with no germinal vesicles were included in the data. In each egg, the longest axis and the axis perpendicular to it were measured (under cover slips supported so that their weight would not distort the eggs) with an ocular micrometer with a magnification of 400 × or 100 ×, depending on size. Egg volume was calculated as that of a prolate spheroid with two axes equal to these measurements. Five eggs were measured from each specimen collected until 1983; in 1984 and 1985, 50 eggs per specimen were measured.

To determine whether size of eggs from each individual was correlated with gonadal content, egg number, or body size, the specimens of *Echinometra lucunter* (Linnaeus), *E. viridis* A. Agassiz and *E. vanbrunti* A. Agassiz collected in 1976–77 were dissected, and the volume of the gonads was measured by displacement in sea water; ovaries were then placed in 0.5 M KCl solution, which caused them to shed their eggs if they were ripe. Formalin was added to the egg suspension and (after vigorous agitation) an aliquot was drawn into a 100- μ l capillary tube, so that eggs could be counted and so that the total number of eggs per female could be estimated (Hinegardner, 1975). The tests, with spines and jaws attached, were dried and weighed.

To determine possible changes in egg volume due to formalin preservation, the eggs of each of 10 females per species collected in 1984 and 1985 were split in two batches. Fifty fresh eggs from the first batch were measured 3 h after shedding. Formalin was added to the second batch at the time of measurement of the first, and 50 eggs were measured at a later date. Subsequent spot measurements of aliquots from the preserved eggs indicated that size does not change appreciably with time in the preservative.

TABLE I

Species, localities, and range of dates sampled. House Reef and Tiantupu are near San Blas Point in the San Blas Archipelago (see Lessios *et al.*, 1984, for map). Isla Taboguilla and Isla Culebra are located off Panama City. The Islas Secas are located in the Gulf of Chiriqui. Isla Margarita (formerly known as Ft Randolph) and Maria Chiquita are located on the Caribbean coast of the Isthmus of Panama. Morbitupu is on the east side of the Salar Salada chain, San Blas Archipelago.

Species	Locality	Time period sampled	Time period sampled in consecutive months	Time period sampled in consecutive 3-day intervals
<i>Eucidaris tribuloides</i>	House Reef (C)	December 1982–June 1985	December 1982–December 1983	–
	Tiantupu (C)	–	–	23 June–29 July 1983
<i>Eucidaris thourarsi</i>	Isla Taboguilla (P)	July 1982–July 1983	July 1982–July 1983	–
<i>Diadema antillarum</i>	Isla Galeta (C)	June 1982–December 1982	September 1982–December 1982	15 September–19 November 1982*
	House Reef (C)	September 1982–October 1985	October 1982–December 1982	15 October–20 December 1982*
<i>Diadema mexicanum</i>	Isla Taboguilla (P)	June 1982–October 1985	September 1982–December 1982	15 September–20 December 1982*
	Isla Secas (P)	5 October 1982	–	–
<i>Lytechinus variegatus</i>	House Reef (C)	July 1982–October 1985	July 1982–May 1983	7 April–7 May 1983
<i>Lytechinus williamsi</i>	House Reef (C)	July 1982–October 1985	July 1982–January 1983	–
<i>Triptenaustes ventricosus</i>	House Reef (C)	November 1980–October 1985	July 1982–May 1983	–
<i>Echinometra lucunter</i>	House Reef (C)	July 1982–October 1985	July 1982–March 1983	–
	Isla Margarita (C)	March 1976–June 1977	March 1976–June 1977	–
	Maria Chiquita (C)	July 1976–June 1977	July 1976–June 1977	–
<i>Echinometra viridis</i>	House Reef (C)	June 1982–September 1985	June 1982–May 1983	7 April–7 May 1983
	Isla Margarita (C)	March 1976–April 1984	March 1976–April 1977	–
<i>Echinometra vanbrunti</i>	Punta Paitilla (P)	June 1976–September 1985	June 1976–June 1977	–
	Isla Culebra (P)	June 1976–September 1980	June 1976–June 1977	–
<i>Clypeaster rosaceus</i>	House Reef (C)	November 1980–September 1985	June 1982–May 1983	–
<i>Clypeaster subdepressus</i>	House Reef (C)	August 1982–November 1982	August 1982–November 1982	–
	Tiantupu (C)	October 1982–October 1985	October 1982–May 1983	–
	Morbitupu (C)	August 1982	–	–
<i>Leodia sexiesperforata</i>	Tiantupu (C)	October 1982–October 1985	October 1982–November 1983	–
	Morbitupu (C)	August 1982	–	–

(C) = Caribbean; (P) = eastern Pacific; * not always sampled every 3 days (see Lessios, 1984, for details of sampling regime).

DATA ANALYSIS

The mean volume of 50 fresh eggs per individual from animals collected on the same day in 1984 and 1985 were compared through ANOVA, followed by Student–Newman–Keuls (S–N–K) multiple range tests to determine which means were significantly different from the rest. This is the only instance in which dimensions of eggs were used directly as raw data. For all other comparisons the mean egg volume from all eggs measured from the same individual was used as the raw datum in the analysis. The term “individual egg volume” is used in this article to refer to this mean volume of eggs from a single mother.

Correlation was used to look for relationships in the 1976–77 data between egg size and egg number, body size, or gonadal content of each individual of *Echinometra*. Only individuals in which all four variables were measured were included in this analysis. The question these data address is whether body weight, gonad per unit body weight, or number eggs are good predictors of egg size. Since the effects of locality and season would be reflected in these variables, data from specimens of each species were pooled regardless of time and place of collection. Bivariate and partial correlation coefficients were calculated to describe the relation between individual egg volume and each of the other variables. Bivariate coefficients are a descriptor of a linear relation between two variables with the rest of the variables ignored. Partial coefficients describe the same relationship with the linear effect of other variables held constant.

In species that spawn during a few months of the year or during a few days of a month, it is impossible to collect egg samples continuous in time. Thus, the more marked the spawning periodicity of a species, the weaker the analysis of temporal trends in egg size. Because of this uncontrolled element in the sampling of eggs, and the resultant sporadic distribution of egg samples over time, formal hierarchical or factorial ANOVA designs could not be used to analyze the data. Instead, the following strategy was used: to analyze temporal variation, the mean individual egg volume from all specimens collected in the appropriate time unit (day or month) was compared with those of other time units through ANOVA. In the search for differences between months, the data were analyzed twice. First, the means of all months for which data were available in each locality were compared through ANOVA. Then, the analysis was repeated with only data for 1976–77 or 1982–83, years in which collections were made at regular intervals. The later analysis was meant to uncover possible seasonal trends in the variation of egg size, and was followed by S–N–K tests. Where no significant differences between months were revealed, data from different years were pooled and compared through ANOVA. In species with significant monthly variation, average individual egg volume of a year could be biased by the inclusion of too many or too few individual egg volumes from a particular month. To avoid this problem, eggs from individuals collected in each month of different years were compared separately. For the same reasons, comparisons between localities in species with significant temporal variation were limited to months sampled in common.

To see if egg size variation depended on the reproductive state of the population at the time of collection, a correlation was sought between egg size and a measure of the reproductive activity of the population at each time unit. The measure of reproductive activity was either the percent of animals (males and females) that spawned in that particular time period when injected with 0.5 M KCl solution (Lessios, 1984, 1985, in prep.) or the mean gonadal index (volume of gonad, divided by body weight, and adjusted for allometry) of the sample (Lessios, 1981, in prep.).

RESULTS

EFFECTS OF PRESERVATION

The average change in egg volume due to preservation varied greatly between species (Table II); in some, the change was minimal; however, in *Leodia sexiesperforata* (Leske), the eggs shrank to half their volume when placed in formalin. Inexplicably, the eggs of two species actually expanded in the preservative. A question that needs to be settled is whether apparent intraspecific variability in egg size might be due to bias introduced by differential shrinkage in the preservative of eggs collected at different times or in different localities. If this were the case, we would expect to find the largest differences between months, days or localities in species whose egg size was affected most severely or most variably by preservation. Such a trend is not evident (Table II). Eggs from species, such as *Echinometra vanbrunti* or *E. lucunter*, that on the average suffered only slight changes in egg volume due to preservation, show higher variability in time and space than eggs from species such as *Leodia sexiesperforata*, *Eucidaris tribuloides* (Lamarck), or *E. thouarsi* (Valenciennes), which were severely affected by preservation. This is not to say that preservation did not introduce artificial variation into the data, but that it is not likely that such variation caused systematic bias in temporal and spatial comparisons. Apparent within-month or within-day variability undoubtedly has increased because of preservation, but this would make significant differences harder to demonstrate.

VARIATION BETWEEN EGGS FROM DIFFERENT INDIVIDUALS

Fig. 1 indicates that there were significant differences between egg volumes from different females in all species, even when the eggs were measured fresh, and even when they came from females collected in the same locality on the same day. The question addressed in the rest of this article is whether this variation between individuals is distributed randomly over time and space.

Given that individuals differed in the size of their eggs, we would like to know whether there was any trend for larger or reproductively more mature individuals to have larger eggs, or whether animals of a given size had more eggs if these eggs were small. The three species of *Echinometra* used to address these questions indicated that such

TABLE II

Temporal and spatial variation in egg volume, reproductive periodicity, and effect of preservation in each species. See text for the manner in which significance of variation in each species was calculated. *F/P* is the mean of ten ratios; each ratio was calculated by dividing the mean volume of 50 fresh eggs from an individual with the mean egg volume of 50 preserved eggs from the same individual (range of ratios in parentheses).

Species	Locality	Significant variation between							Mode of spawning		<i>F/P</i>
		Individuals in same day	Days within month	Months	Years	Localities	Lunar	Seasonal			
<i>Eucidaris tribuloides</i>	House Reef	Yes	-	No	No	No	No	Yes	Yes	1.25 (0.89-1.47)	
	Tiantupu	-	Yes	No	-	-	-	-	-	-	
<i>Eucidaris thouarsi</i>	Islas Taboguilla	Yes	-	No	No	-	-	?	No	1.16 (1.01-1.86)	
<i>Diadema antillarum</i>	Punta Galeta	-	Yes	No	-	No	No	Yes	No	-	
	House Reef	Yes	Yes	No	No*	-	-	-	Yes	0.98 (0.90-1.09)	
<i>Diadema mexicanum</i>	Isla Taboguilla	Yes	No*	No	No*	No*	-	Yes	Yes	1.05 (0.95-1.13)	
	Isla Secas	-	-	-	-	-	-	-	-	-	
<i>Lytechinus variegatus</i>	House Reef	Yes	Yes	Yes	No	-	-	Yes	No	1.12 (0.93-1.48)	
<i>Lytechinus williamsi</i>	House Reef	Yes	-	Yes	Yes	-	-	No	Yes	1.15 (0.94-1.81)	
<i>Tripneustes ventricosus</i>	House Reef	Yes	-	Yes	Yes	-	-	No	No	0.99 (0.93-1.05)	
<i>Echinometra lucunter</i>	House Reef	Yes	-	No	Yes	Yes ⁺	-	No	No	1.02 (0.94-1.07)	
	Isla Margarita	-	-	No	-	-	-	-	-	-	
	Maria Chiquita	-	-	No	-	-	-	-	-	-	
<i>Echinometra viridis</i>	House Reef	Yes	No	Yes	Yes	No ⁺ *	Yes	No	Yes	1.0 (0.96-1.08)	
	Isla Margarita	-	-	No*	-	-	-	-	-	-	
<i>Echinometra vanbrunni</i>	Punta Patilla	Yes	-	No*	Yes	No*	-	?	Yes	1.07 (1.00-1.12)	
	Isla Culebra	-	-	Yes	-	-	-	-	-	-	
<i>Clypeaster rosaceus</i>	House Reef	Yes	-	Yes	Yes	-	Yes	Yes	Yes	1.24 (1.03-1.99)	
<i>Clypeaster subdepressus</i>	House Reef	-	-	Yes	-	-	-	No	No	-	
	Tiantupu	Yes	-	Yes	Yes	Yes	-	Yes	Yes	1.09 (0.85-1.58)	
	Morbitupu	-	-	-	-	-	-	-	-	-	
<i>Leodia sexiesperforata</i>	Tiantupu	Yes	-	No	No	No	No	No	Yes	2.29 (1.40-3.47)	
	Morbitupu	-	-	-	-	-	-	-	-	-	

* Lack of significant differences likely to be due to small sample size. + Data collected in different localities at different times.

the result of pooling individuals from two localities; the same analysis was carried out separately for each locality with similarly nonsignificant results.

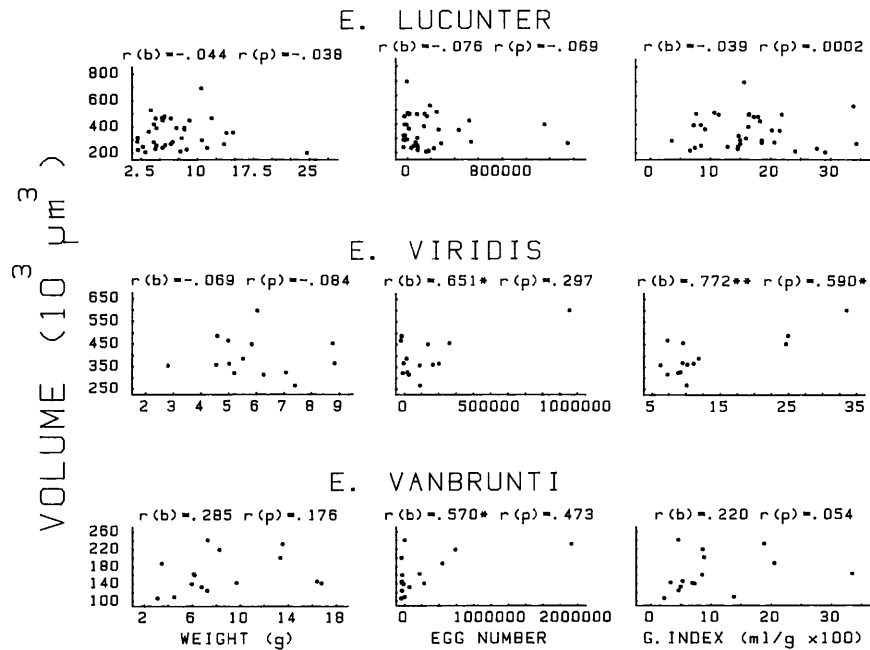


Fig. 2. *Echinometra lucunter*, *E. viridis*, *E. vanbrunti*: relation between individual egg volume and body weight, egg number, and gonadal index (ml of gonadal tissue, divided by body weight) in animals collected between October 1976 and April 1977 in Isla Margarita (*E. lucunter*, *E. viridis*), Maria Chiquita (*E. lucunter*), Punta Paitilla (*E. vanbrunti*), and Isla Culebra (*E. vanbrunti*). Bivariate [$r(b)$] and partial [$r(p)$] correlation coefficients are shown at the top of each graph. Samples of each species from different localities are pooled. Significance of correlation coefficients: * $P < 0.05$, ** $P < 0.001$.

VARIATION WITHIN MONTHS

Only five species (four from the Caribbean, one from the eastern Pacific) provided a sufficient number of eggs on enough 3-day intervals to allow analysis of possible variation in egg size within the lunar month.

In *Eucidaris tribuloides* (Fig. 3) daily means of individual egg volume differ significantly ($F = 4.108$, $P < 0.001$). Eggs contained by the sea urchins immediately after full moon were larger than eggs contained during the rest of the time, but more lunar cycles must be sampled to determine whether this is a real pattern. The correlation between percent of animals spawning on a given day and mean individual egg volume of that day was not significant (Spearman rank correlation, $r_s = -0.543$, $P > 0.05$).

Diadema antillarum Philippi (Fig. 3) was not sampled every 3 days, except in June and September at Galeta. This is a species that spawns exclusively around new moon

(Lessios, 1984), so sampling effort and available data on egg size were concentrated on this time. At House Reef samples were taken every day between 3 days before and 4 days after new moon; animals were also injected with KCl solution 2 days after full moon. Individual egg volumes for each spawning cycle from one full moon to the next

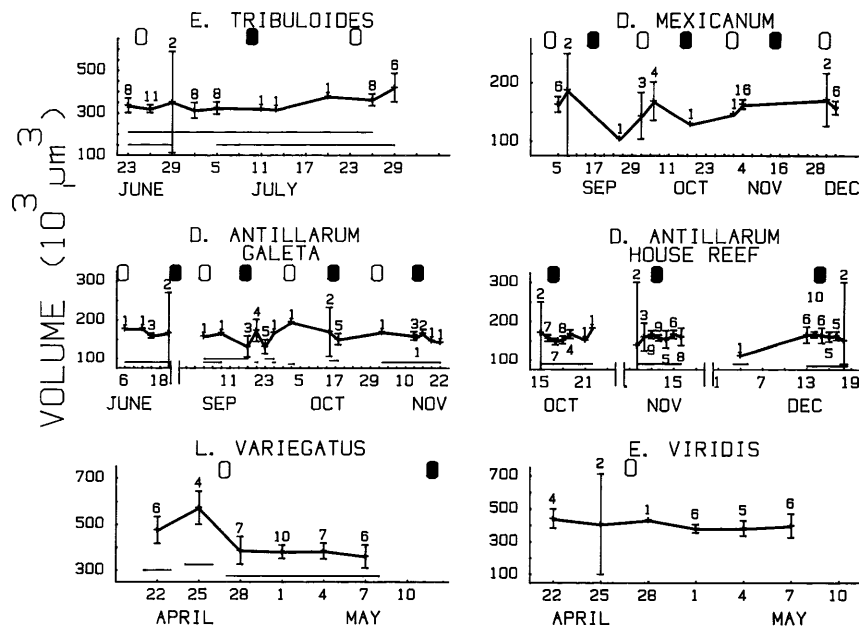


Fig. 3. Number of individuals, daily means, and 95% confidence intervals of individual egg volumes in *Eucidaris tribuloides* (collected at Tiantupu in 1983), *Diadema mexicanum* (collected at Isla Taboguilla in 1982), *D. antillarum* (collected at Isla Galeta and House Reef in 1982), *Lytechinus variegatus* (collected at House Reef in 1983), and *Echinometra viridis* (collected at House Reef in 1983). Phases of the moon are shown at the top of each graph. In species with significant daily variation, lines on the same level parallel to the x axis indicate means that are not significantly different on the 0.05 *P* level on the basis of an S-N-K test. In *D. antillarum* each spawning cycle from full moon to one day before the next full moon was analyzed separately; in *D. mexicanum* each cycle from new moon to one day before the next new moon was analyzed separately.

were analyzed separately. At Galeta, there were no significant differences between daily means in June ($F = 0.616$, $P = 0.650$), but there were significant differences in September ($F = 5.408$, $P = 0.012$), October ($F = 7.788$, $P = 0.029$), and (marginally, so that it cannot be detected by the S-N-K test) November ($F = 9.336$, $P = 0.048$). At House Reef no significant differences between daily means existed in October ($F = 2.437$, $P = 0.050$), or November ($F = 1.048$, $P = 0.412$), but there were significant differences in December ($F = 2.564$, $P = 0.035$), a month in which one female with a few eggs could be found shortly after full moon. Thus, where the full lunar cycle was sampled and where sample sizes were not miniscule (June at Galeta), significant

differences in daily means of individual egg volume were found. This variation, however, displayed no obvious, repeatable pattern from month to month. What is more, despite the large number of available days with which to calculate the correlation, no significant relation was found between the percent of animals spawning on a given day and the mean individual egg volume of that day (Galeta: $r_s = -0.133$, $P > 0.05$; House Reef: $r_s = -0.048$, $P > 0.05$). Thus, changes in egg size of this species do not seem to be determined by the reproductive state of the population containing the eggs.

The eastern Pacific species *Diadema mexicanum* A. Agassiz (Fig. 3) also reproduces with a lunar cycle, but spawning is concentrated around full moon (Lessios, 1984). Individuals were sampled every 3 days in September but only shed eggs in 2 of these days. Sampling was more sporadic in the other 3 months (see Lessios, 1984). Each spawning cycle from one new moon to the next was analyzed separately. There were no significant differences between daily means of individual egg volume in any of the cycles ($F = 3.539$, $P = 0.109$; $F = 5.028$, $P = 0.064$; $F = 2.951$, $P = 0.083$; $F = 2.803$, $P = 0.145$ for successive cycles). However, there was a significant correlation between the percent of animals spawning on a given day and the mean individual egg volume of that day ($r_s = 0.724$, $P < 0.05$). Individual egg volume reached a peak right after full moon, at the same time that the majority of the individuals in the population were spawning.

Lytechinus variegatus (Lamarck) (Fig. 3), another species that tends to spawn with lunar periodicity (Lessios, in prep.), also showed significant differences between daily means ($F = 13.456$, $P < 0.001$). However, the correlation between percent of animals spawning on a given day and the mean individual egg volume of that day was not significant ($r_s = -0.657$, $P > 0.05$). Peak spawning in this species occurs after full moon, but the largest mean individual egg volume is reached before this time.

Echinometra viridis (Fig. 3), a species in which spawning definitely does not follow a lunar cycle (Lessios, in prep.), showed no significant differences between daily means of individual egg volume ($F = 1.151$, $P = 0.370$). Egg size of this species fluctuated very little with days of the month. No significant correlation existed between percent of animals spawning and mean individual egg volume of each day ($r_s = -0.614$, $P > 0.05$).

VARIATION BETWEEN MONTHS, BETWEEN YEARS, AND BETWEEN LOCALITIES

Monthly variation could be analyzed for all 13 species. For the sake of brevity, data on yearly and spatial variation are also presented in this section.

In *Eucidaris tribuloides* (Fig. 4) there were no significant differences between monthly means at House Reef ($F = 1.852$, $P = 0.090$ for all data; $F = 1.632$, $P = 0.250$ for 1982–83 data) or in Tiantupu ($F = 0.035$, $P = 0.852$). No significant correlation existed between mean gonadal index in 1982–83 and mean individual egg volume of each month ($r_s = 0.657$, $P > 0.05$, data from both localities). Though data from a single locality did not span an entire year, Tiantupu and House Reef are sufficiently close to each other (≈ 500 m) to lead to the expectation that if a seasonal cycle in egg size existed, animals

on both reefs would have conformed to it. Following this logic, we can tentatively conclude that this species shows no seasonal variation in egg size. In fact, there were no significant differences between mean individual egg volumes from House Reef and Tiantupu ($F = 0.035$, $P = 0.852$, data for each locality pooled), which would indicate that neither distance nor different times of collection produced any systematic variation between the two localities (unless the two sources of possible variation cancel each other). There were no significant differences between years at House Reef ($F = 1.322$, $P = 0.254$, data for each year pooled).

Sample size for each month in the eastern Pacific *Eucidaris thouarsi* (Fig. 4) were small, and eggs could be obtained in only half of the months of the year, so the lack of significant differences between monthly means is not particularly meaningful. It can only be said that large changes in individual egg volume did not occur between July and November 1982. No significant correlation existed between mean gonadal index and mean individual egg volume of each month ($r_s = 0.257$, $P > 0.05$).

In *Diadema antillarum* (Fig. 4) there were no significant differences between monthly means at House Reef ($F = 1.258$, $P = 0.283$ for all data; $F = 0.935$, $P = 0.427$ for 1982

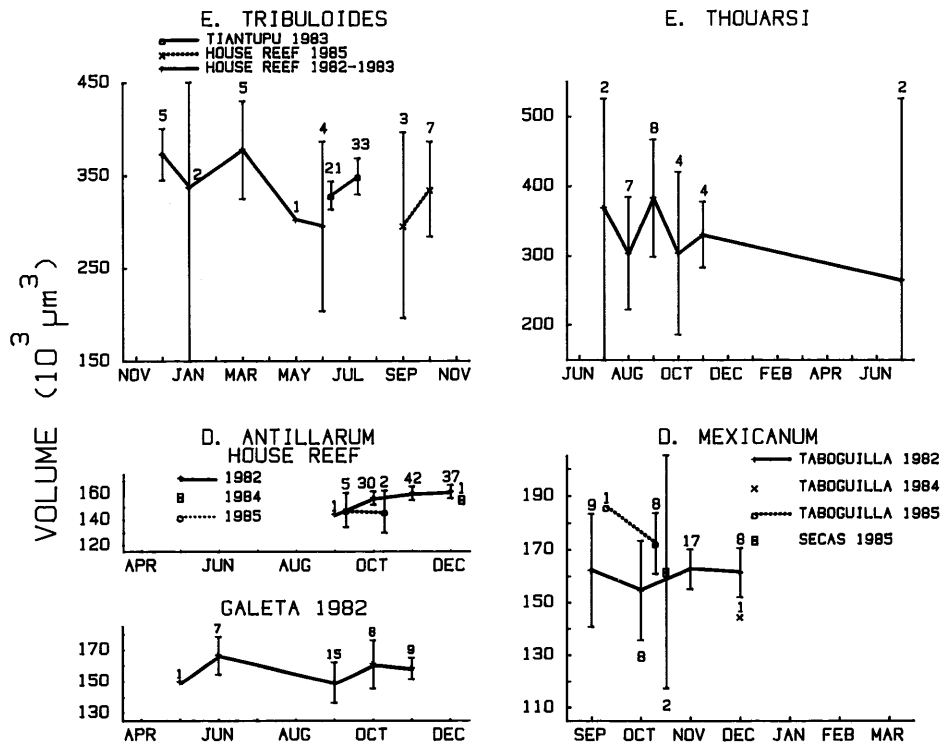


Fig. 4. Number of individuals, monthly means, and 95% confidence intervals of individual egg volumes in *Eucidaris tribuloides* collected at two localities in the San Blas archipelago, *Eucidaris thouarsi* collected at Isla Taboguilla, Bay of Panama, in 1982-83, *Diadema antillarum* collected at two localities in the Caribbean, and *Diadema mexicanum* collected at two localities in the eastern Pacific.

data) or at Isla Galeta ($F = 1.326$, $P = 0.280$). Because significant within-month variability existed in this species (Fig. 3), the analysis was repeated with data obtained only from 2 days before to 5 days after new moon to guard against the possibility that between-month variability was masked by daily fluctuations. Again, individual egg volume did not vary significantly between months (House Reef $F = 1.437$, $P = 0.235$, $n = 126$; Galeta: $F = 0.079$, $P = 0.924$, $n = 21$). Because means based on more than one individual were available for only 4 months of the year, correlations between reproductive state of the population and size of eggs were not statistically possible. Lack of significant variability extended to comparisons between years and between localities. There were no significant differences between years at House Reef ($F = 2.349$, $P = 0.100$, data for each year pooled) or between House Reef and Galeta ($F = 1.104$, $P = 0.296$, data for each locality pooled).

The eastern Pacific *Diadema mexicanum* (Fig. 4) showed the same lack of major fluctuations as its Caribbean congener. In both species mean individual egg volume did not vary by $> 20\,000\ \mu\text{m}^3$. There were no significant differences between available monthly means in Taboguilla ($F = 1.077$, $P = 0.390$ for all data; $F = 0.348$, $P = 0.791$ for 1982 data). *D. mexicanum* is only reproductively active between August and December (Lessios, 1981). Therefore, the entire reproductive period was sampled. Though eggs collected in 1985 appear somewhat larger than those collected previously, there were no significant differences between years in Taboguilla ($F = 2.519$, $P = 0.091$, data for each year pooled). The two specimens from Secas Islands, 500 km away, did not differ significantly in individual egg volume from those of Taboguilla ($F = 0.014$, $P = 0.907$, data for each locality pooled).

In contrast to the species discussed thus far, *Lytechinus variegatus* (Fig. 5) showed between-month variation. Eggs were large from July to September, and then gradually diminished in size. There were significant differences between monthly means ($F = 9.234$, $P < 0.001$ for all data; $F = 11.349$, $P < 0.001$ for 1982–83 data). Specimens in 1982–83 were collected 2–4 days before new moon in all months except April and May (the months in which daily variation was studied). It is fair to question whether significant variation within these 2 months (Fig. 3) might have biased their mean values relative to other months. This is not the case. If only data collected 2–4 days before new moon in April and May 1983 are included in the analysis, the differences between monthly means remain significant ($F = 7.860$, $P < 0.001$ for 1982–83 data). *L. variegatus* reproduces throughout the year, with only slight fluctuations in the number of animals spawning each month (Lessios, 1985). Accordingly, no significant correlation could be found between the percent of animals spawning and the mean individual egg volume of each month ($r_s = -0.533$, $P > 0.05$). Data from other years agree with those obtained in 1982–83: there were no significant differences between September 1982 and September 1985 mean individual egg volume ($F = 2.287$, $P = 0.161$), between December 1982 and December 1984 ($F = 0.889$, $P = 0.373$), or (more meaningfully) between October 1982 and October 1985 ($F = 2.075$, $P = 0.172$).

Egg size in *Lytechinus williamsi* Chesher (Fig. 5) also appears to follow a pattern of

gradual changes, with peak values in July and August, then a decline until December. There were significant differences between monthly means ($F = 7.642$, $P < 0.001$ for all data; $F = 6.423$, $P < 0.001$ for 1982–83 data). Reproductive activity in this species decreases in December and January (Lessios, 1985), but no significant correlation

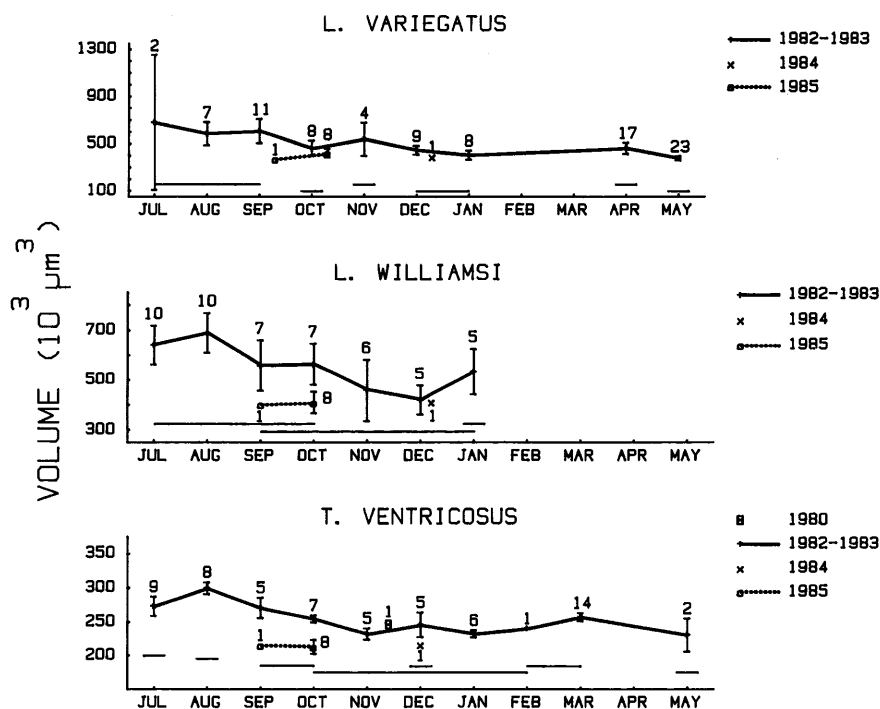


Fig. 5. Number of individuals, monthly means, and 95% confidence intervals of individual egg volumes in *Lytechinus variegatus*, *Lytechinus williamsi*, and *Tripneustes ventricosus* collected at House Reef. Lines on the same level parallel to the x axis indicate 1982–83 means that are not significantly different on the 0.05 P level on the basis of an S–N–K test.

existed between the percent of animals spawning and mean individual egg volume of each month ($r_s = 0.536$, $P > 0.05$). There were no significant differences between September 1982 and September 1985 mean individual egg volume ($F = 1.810$, $P = 0.227$), or between December 1982 and December 1984 ($F = 0.040$, $P = 0.852$). In contrast, eggs obtained in October 1985 were significantly smaller than those collected in October 1982 ($F = 17.915$, $P = 0.001$), suggesting that the 1982–83 pattern of monthly egg size variation may not repeat itself on the same level every year.

Gradual changes over time also occur in *Tripneustes ventricosus* (Lamarck) (Fig. 5). Like the two species of *Lytechinus*, *T. ventricosus* showed a decline in egg size after September. There were significant differences between monthly means ($F = 25.846$, $P < 0.001$ for all data; $F = 22.750$, $P < 0.001$ for 1982–83 data). This species spawns

throughout the year (Lessios, 1985). No significant correlation existed between percent of animals spawning and mean individual egg volume of each month ($r_s = 0.624$, $P > 0.05$). There were no significant differences between November 1980 and November 1982 mean individual egg volume ($F = 3.779$, $P = 0.124$) or between December 1982 and December 1984 ($F = 3.201$, $P = 0.148$); however, 1985 means were significantly smaller than those of 1982 (September: $F = 17.469$, $P = 0.014$; October: $F = 66.845$, $P < 0.001$).

Echinometra lucunter (Fig. 6), a species without any defined reproductive periodicity in Panama (Lessios 1981, 1985), showed only slight changes through time in egg size. There were no significant differences between monthly means in Isla Margarita ($F = 0.366$, $P = 0.970$), Maria Chiquita ($F = 1.569$, $P = 0.213$), or in 1982–83 at House Reef ($F = 1.791$, $P = 0.121$). No significant correlation existed at House Reef between the percent of animals spawning and mean individual egg volume of each month ($r_s = -0.217$, $P > 0.05$). However, there were significant differences between years at House Reef ($F = 4.398$, $P = 0.009$, data for each year pooled, 1982–83 series considered

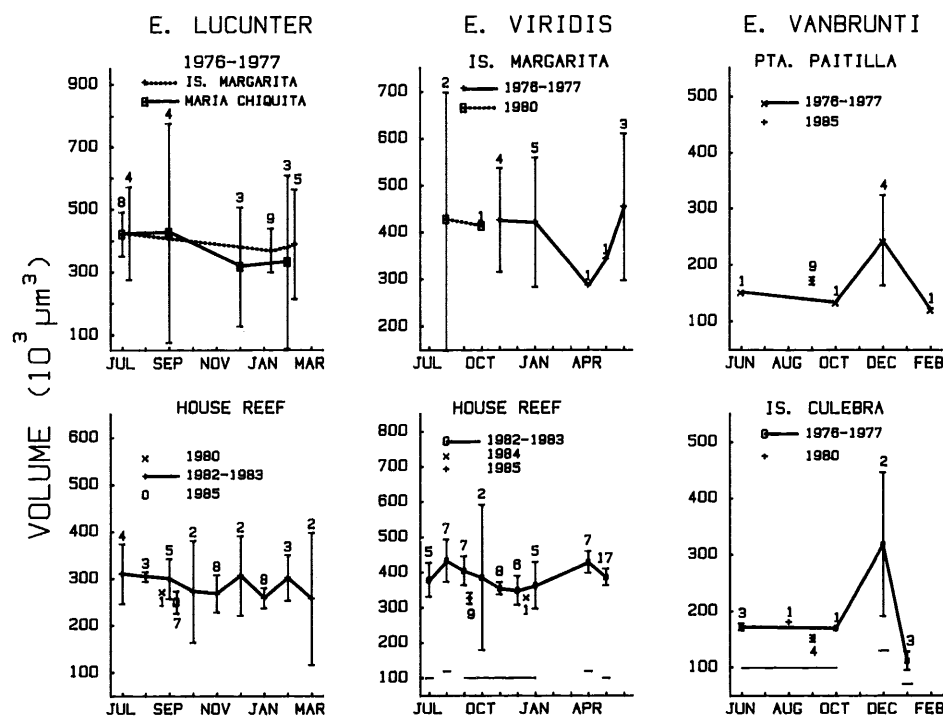


Fig. 6. Number of individuals, monthly means, and 95% confidence intervals of individual egg volumes in *Echinometra lucunter* and *E. viridis* collected at three localities in the Caribbean, and *E. vanbrunti* collected at two localities in the Bay of Panama. In species with significant monthly variation, lines on the same level parallel to the x axis indicate 1982–83 or 1976–77 means that are not significantly different on the 0.05 P level on the basis of an S–N–K test.

as same year; on an S–N–K test, 1985 and 1980 differed at the 0.05 P level from 1982). When mean individual egg volumes from Maria Chiquita and Isla Margarita (16 km away from each other) were compared, no significant differences emerged ($F = 0.177$, $P = 0.676$). Addition of House Reef (110 km from Maria Chiquita) to the comparison produced significant differences between localities ($F = 19.045$, $P < 0.001$, data for each locality were pooled; on an S–N–K test, Maria Chiquita and Isla Margarita differed at the 0.05 P level from House Reef). Because data at House Reef were not gathered at the same time as the other two sites it is not clear whether these differences were due to temporal or spatial variation.

Echinometra viridis (Fig. 6), unlike its sympatric congener, shows reproductive periodicity at both Isla Margarita (Lessios, 1981) and at House Reef (1985). Small sample sizes led to no detection of monthly differences in individual egg volume at Isla Margarita ($F = 0.608$, $P = 0.720$ for all data; $F = 0.835$, $P = 0.536$ for 1976–77 data), but significant differences between months were found at House Reef ($F = 4.758$, $P < 0.001$ for all data; $F = 3.238$, $P = 0.004$ for 1982–83 data). There are no abrupt changes from month to month at House Reef, and two peaks occur, one in August and one in April. August is also the time of peak reproductive activity at this locality, but April is a time of relative quiescence in reproduction (Lessios, 1985). No significant correlation existed at House Reef between the percent of animals spawning and mean individual egg volume of each month ($r_s = 0.267$, $P > 0.05$), or between monthly gonadal index and mean individual egg volume at Isla Margarita ($r_s = 0.450$, $P > 0.05$). There were no significant differences between December 1982 and December 1984 at House Reef ($F = 0.160$, $P = 0.706$), but there were significant differences between September 1982 and September 1985 ($F = 22.160$, $P < 0.001$). Isla Margarita and House Reef were sampled in different years, but a comparison between months sampled in common shows no significant differences ($F = 0.837$, $P = 0.364$, data pooled for each locality).

Sample sizes in *Echinometra vanbrunti* (Fig. 6) were small, yet the distinct December peak in individual egg volume produced generally significant differences. Though the differences between 1976–77 monthly means at Punta Paitilla were not significantly different ($F = 2.662$, $P = 0.221$) by themselves, an F value of 7.603 ($P = 0.003$) was obtained when September 1985 was included in the analysis. An S–N–K test showed differences at the 0.05 P level between December 1976 and the rest of the months. At Isla Culebra, despite small sample sizes, the differences between monthly means were significant ($F = 263.795$, $P < 0.001$ for all data; $F = 292.882$, $P < 0.001$ for 1976–77 data). Because egg size in the two localities changed in parallel over time, data used to compare them were pooled despite significant temporal variation at Isla Culebra. No significant differences were found between Punta Paitilla and Isla Culebra ($F = 0.185$, $P = 0.670$ for all data; $F = 3.856$, $P = 0.121$ for 1976–77 data). However, means for September 1980 at Isla Culebra and September 1985 at Punta Paitilla were significantly different ($F = 19.842$, $P = 0.001$); whether this represents spatial or between-year variation cannot be said. December represents the end of the breeding season of this species (Lessios, 1981). Though correlations between egg size and degree of spawning

are not statistically valid with the number of months for which data are available, it is clear that the largest eggs are not produced at the time of peak spawning activity.

Clypeaster rosaceus (Linnaeus) (Fig. 7) remains reproductively quiescent from January to April (Lessios, 1985), so the 7 months included in the data represent the entire

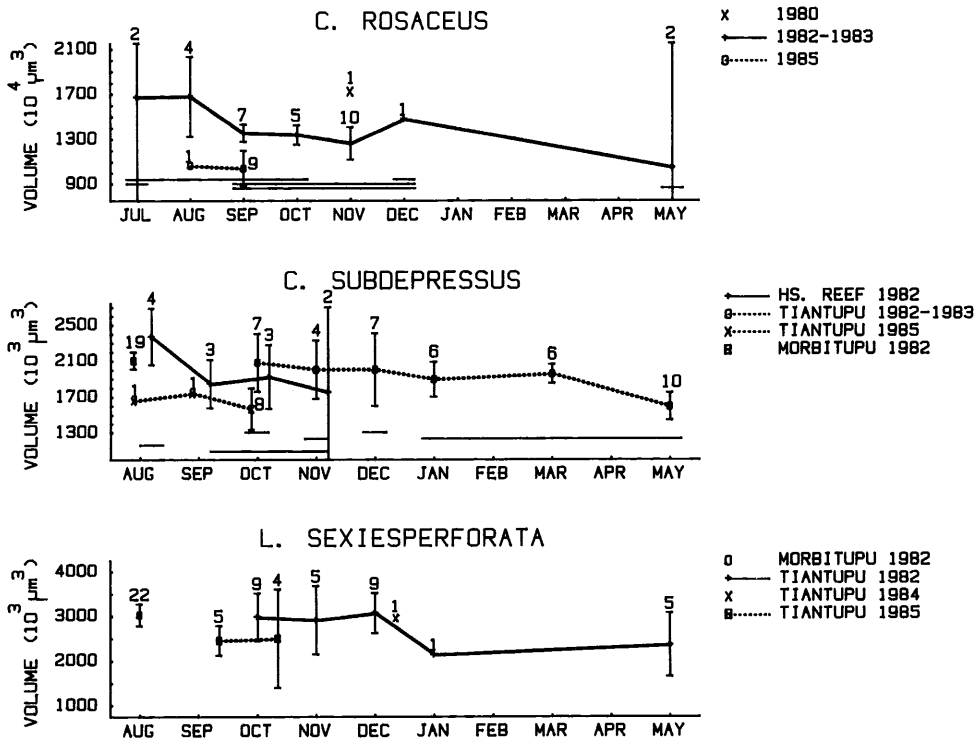


Fig. 7. Number of individuals, monthly means, and 95% confidence intervals of individual egg volumes in *Clypeaster rosaceus* collected at House Reef, and in *C. subdepressus* and *Leodia sexiesperforata* collected at three localities in the San Blas archipelago. In species with significant monthly variation, lines on the same level parallel to the x axis indicate 1982-83 means that are not significantly different on the 0.05 P level on the basis of an S-N-K test. In *C. subdepressus* monthly means were compared separately for each locality. Note order of magnitude difference in the y axis scale of *C. rosaceus*.

breeding season of this species. There were significant differences between monthly means ($F = 5.970, P < 0.001$ for all data; $F = 4.602, P = 0.003$ for 1982-83 data). Like the species of *Lytechinus* and *Tripneustes*, *C. rosaceus* had smaller eggs from September to December. Peak reproductive activity in *C. rosaceus* occurs in September (Lessios, 1985), so the correlation between percent of animals spawning and mean individual egg volume of each month is not significant ($r_s = 0.643, P > 0.05$). There are no significant differences between November 1980 and November 1982 ($F = 4.624, P = 0.060$) or between August 1982 and August 1985 ($F = 6.074, P = 0.091$), but there are significant differences between September 1982 and September 1985 ($F = 14.285, P = 0.002$).

Smaller eggs after September were also present in *Clypeaster subdepressus* (Gray) (Fig. 7). There were significant differences between monthly means (Tiantupu: $F = 3.314$, $P = 0.005$ for all data; $F = 3.358$, $P = 0.014$ for 1982–83 data; House Reef: $F = 6.169$, $P = 0.018$). However, this pattern does not appear consistent between years. There were significant differences at Tiantupu between October 1982 and October 1985 ($F = 11.550$, $P = 0.004$). *C. subdepressus* reproduces throughout the year (Lessios, 1985), and there was no significant correlation between percent of animals spawning and mean individual egg volume of each month ($r_s = -0.179$, $P > 0.05$, data from House Reef and Tiantupu pooled). There were significant differences between August mean individual egg volumes at House Reef and Morbitupu, 29 km away ($F = 6.159$, $P = 0.022$), but there were no differences between common month means at House Reef and Tiantupu (October: $F = 0.556$, $P = 0.477$, November: $F = 1.219$, $P = 0.332$, data of two months pooled for each locality: $F = 1.733$, $P = 0.209$).

Leodia sexiesperforata (Fig. 7) shows a very sharp decline in reproductive activity between December and January, from which it recovers by May (Lessios, 1985). Within-month variability in egg size of this species was high, so that no significant trend of temporal variability could be shown. There were no significant differences between monthly means ($F = 1.236$, $P = 0.272$ for all data; $F = 1.474$, $P = 0.241$ for 1982–83 data), no significant differences between October 1982 and October 1985 ($F = 1.268$, $P = 0.284$) or between years ($F = 1.355$, $P = 0.271$, data of each year pooled, 1982–83 series considered as the same year). Spatial variation was also not significant; mean individual egg volumes were very similar at Morbitupu and at Tiantupu ($F = 2.454$, $P = 0.123$, data for each locality pooled). Since monthly means vary little and eggs were obtained in months when 90–100% of the animals were spawning, no significant correlation could be found at Tiantupu between percent of animals spawning and mean individual egg volume of that month ($r_s = 0.267$, $P > 0.05$).

DISCUSSION

Several limitations are inherent in the data. Formalin-preserved eggs were used in all analyses, except for the comparisons of egg size from different individuals collected in the same day (Fig. 1). Measurements of fresh and preserved eggs from the same individual indicate that preservation affects the size of echinoid eggs, and that it does so to a different extent in each species; what is more, the degree to which eggs from different individuals collected in the same day shrink or expand in the preservative can vary (Table II). This undoubtedly introduced artificial variation into the data. However, it is assumed that this artificial variation is randomly distributed between compared groups, and thus does not distort the comparisons in the direction of significant differences in mean egg size. Two lines of indirect evidence support this assumption. (1) In the comparison of fresh eggs from individuals collected in the same day, significant differences were found, suggesting that at least some of the variability is not caused by

preservation. (2) Species, such as *Leodia sexiesperforata*, whose eggs were more severely or more variably affected by preservation did not show more apparent variability between daily, monthly or yearly means (Table II). Thus, preservation may have exaggerated the amount of within-group apparent variability, but this artifact has simply made significant differences between group means harder to demonstrate statistically. If this is correct, lack of significant differences between mean egg size of individuals collected in different days, months, years, or localities, should not be interpreted as meaning that eggs of these species are necessarily less variable between groups than eggs of species in which significant differences were found.

Variable sample size is another reason for viewing lack of significant differences with caution. Though lack of significant variation never means that real differences do not exist, it is often necessary to pretend that it does if the sample size appears convincing. In the analyses carried out in this paper there are two facets to sample size. Number of individuals sampled at each time unit was lower in some species, thus making the degree of between-group difference required for significance in the ANOVA comparison vary. Number of time units sampled also varied between species, so that correlations between reproductive state at a particular time and size of eggs produced at that time vary in reliability.

A final limitation of the study is that the distance between localities was not constant in all species, and it was always very small compared to the range of the species. Thus, lack of statistically significant spatial variation in some species is inconclusive as to whether populations in the entirety of its range are geographically variable in egg size.

Because of the confounding factors mentioned above, a search for pattern through comparisons between species has to be conducted carefully. Nevertheless, it is worth asking whether there are any patterns shared by all species studied, and whether there is any trend for species that reproduce periodically to be more (or less) likely to show temporal variation in egg size than species that remain constantly ripe. A summary of egg size variation in each species along with the distribution of its reproduction over time is presented in Table II. In all 13 of the species, mean egg sizes from different individuals collected on the same day were significantly different, showing that there is more variability between eggs from different mothers than eggs from the same mother. That different individuals produce eggs of different size has been demonstrated before in echinoids (Goldforb, 1935; Turner & Lawrence, 1979); however, it is worth pointing out that the individuals compared in this study were collected within a few meters of each other at the same time, yet the volume of their eggs still could vary by a factor of 2 (*Eucidaris tribuloides*) or 3 (*Leodia sexiesperforata*). Either egg size is genetically polymorphic, or it reflects some reproductive, developmental, or nutritional attribute of the individual that produced the eggs.

Three out of the five species studied for daily variation showed significant differences between means, demonstrating that variability between individual egg volumes is not randomly distributed through time. It cannot be deduced from the data at hand whether individuals with different egg size tend to spawn on different days or whether the same

individual may contain eggs of different size depending on the day. However, the present data identify species, such as *Lytechinus variegatus*, that would be good material for an experimental inquiry into this question.

Of the 13 species studied, seven showed significant monthly variation in egg size in at least one locality. Since lack of significant differences is not necessarily indicative of lack of real differences, it is probably correct to conclude that variation within the year is common in echinoid egg size. All the Caribbean species that exhibited significant differences between months show a decline in egg size starting in September 1982. This is a pattern most evident in the Toxopneustidae (*Lytechinus variegatus*, *L. williamsi*, and *Tripneustes ventricosus*) and the Clypeasteridae (*Clypeaster rosaceus* and *C. subdepressus*), but it is also present in *Echinometra viridis*. The length of the period of decline varies between species. Whether such variation can be called seasonal is an open question. Seasonal variation should show an increase to a single annual peak, and should be repeatable from year to year. The data are too limited to examine variation over an entire annual cycle, let alone several cycles.

Temporal changes in egg size, if they corresponded to changes of local environmental parameters, such as temperature, salinity or food availability, should have occurred during the transitions between dry and wet seasons. Marine environments on both sides of the Isthmus of Panama experience seasonal changes in temperature, salinity, and turbidity as the result of the onset of the dry season in late December (Glynn, 1972). Dry season, which lasts until April, affects patterns of reproductive periodicity of Panamanian echinoderms (Hendler 1979; Lessios, 1981, 1985); yet it seems to have no effect on egg size, which would constitute indirect evidence against any hypothesis that would invoke change in local environmental parameters to explain temporal changes in egg size.

Table II indicates that species with eggs that vary in size from month to month are also likely to vary from year to year. There is a statistically significant trend among species for association between significant variability in month and year comparisons (Fisher's exact test $P = 0.029$). This association could well be an artifact of variable sample size, but it further detracts from any claim that monthly variability (where found) follows any predictable seasonal trend.

As pointed out, absence of significant spatial variability does not necessarily mean that egg size is constant throughout the range of the species. However, it is interesting that when temporal variability is taken into account, only *Clypeaster subdepressus* out of a total of eight species had significant differences in egg size between populations. Information from previous studies (Lindahl & Runnstrom, 1929; Lönning & Wennerberg, 1963; Hagström & Lönning, 1967) has been cited as evidence for spatial variability in echinoid egg size (Turner & Lawrence, 1979; Emler *et al.*, in press), though eggs from different localities had probably been collected at different times (the cited articles do not mention the time of collection). The data from Panamanian echinoids suggest that such variation could very possibly be temporal rather than spatial.

An association between mode of spawning and patterns of temporal egg size variation

might have been expected. Species with better synchronized spawning might have also shown more synchrony in changes of egg size through time. However, no association between spawning rhythmicity and temporal variability can be validated by comparisons between species. There is no tendency for species spawning with a lunar rhythm to have (or to not have) significant differences between daily means of individual egg volume or for species with annual reproductive periodicity to show a pattern of significant differences between monthly means, or even for species with any sort of periodicity in spawning to have any sort of significant temporal variation in egg size (Fisher's exact tests $P = 1$).

The various correlations attempted in this paper did not shed much light in the causes of variability of egg size between individuals, but they at least serve in eliminating some factors as unlikely. The general lack of correlation between size of eggs produced at a particular time and reproductive state of the population could be due to small sample sizes or uncontrolled variables, but it still suggests that no simple relation exists between reproductive state and egg size. Unlike oocytes, meiotically and vitellogenically ripe eggs sampled by KCl injections do not seem to vary in size solely because they were caught at different stages of maturing towards a terminal value, constant for the species. The 1976–77 correlative data from *Echinometra lucunter* and *E. vanbrunti* (Fig. 3) confirm this lack of correspondence between gonadal ripeness and size of eggs produced. The significant correlation between egg size and gonadal content in *E. viridis* (Fig. 3) and between daily mean egg size and percent of animals spawning in *D. mexicanum* would suggest the opposite, i.e., in these species, differences in egg size between individuals are indeed the result of differences in reproductive states. However, given the number of correlations attempted in this study, some spurious ones are a distinct possibility. Weight of the mother, though related to the volume of its coelomic cavity, did not correlate well with the size of eggs, nor was there a relationship between number and size of eggs carried by a female. As in fish (Ware, 1975), echinoid egg size may change through time, but, in contrast to fish (Bagenal, 1971; Mann & Mills, 1985), the changes do not appear to have anything to do with the size of the mother.

It is clear that the data presented here are of limited value in explaining the causes of intraspecific variation of echinoid egg size. However, they do establish that temporal variation of considerable magnitude exists, and, given the lack of information about such variation in echinoids (Emlet *et al.*, in press), this is useful knowledge. Relations between egg size and organic content (Strathmann & Vedder, 1977; Lawrence *et al.*, 1984), rate of zygote cleavage (Sato & Maruyama, 1977), larval developmental time (Amy, 1983; McEdward, 1986a), larval size (McEdward, 1986b), and feeding capability (McEdward, 1986b) have been sought in interspecific comparisons, usually based on eggs of a few individuals per species collected at a few times of the year. Searches for patterns between species have the distinct disadvantage that they must assume that eggs from different species are equivalent in all variables except the ones under investigation (Grant, 1983). This unsafe assumption can be avoided if the intraspecific variation demonstrated here is exploited to study the consequences of egg size on early life history parameters.

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