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REPRODUCTIVE VARIABILITY IN LYTECHINUS VARIEGATUS (ECHINODERMATA: ECHINOIDEA) FROM DIFFERENT HABITATS IN A FLORIDA WEST COAST ESTUARY

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's Thesis of

Robert G. Ernest

with a major in Marine Science
has been approved by the Examining Committee
on July 12, 1979 as satisfactory for the
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degree.

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by

Robert G. Ernest

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INTRODUCTION

Lytechinus variegatus (Lamarck, 1916) is a sea urchin inhabiting shallow sub-littoral environments from Beaufort, North Carolina to southern Brazil. Although it has been collected extensively for embryological material in the southeastern United States (Harvey, 1956; Brookbank, 1968). there have been relatively few studies of its reproduction. Gonad indices were used to follow its annual reproductive cycle at Bermuda and Miami (Moore et al., 1963; Moore and Lopez, 1972), while elsewhere, only observations of spawning have been reported (Boolootian, 1966).

In the Anclote estuary near Tarpon Springs, Florida, Lytechinus is the most conspicuous of the macroepibenthic invertebrates, and where requisite environmental conditions are satisfied, adults are found in relatively large numbers. Accordingly, an investigation was initiated to provide information on the reproductive cycle of this species along the west coast of Florida. Because Lytechinus occupies a variety of habitats in the Anclote estuary, one aspect of the study was to determine variability in reproductive activities associated with environmental heterogeneity. Additionally, reproduction was followed histologically so that gametogenesis, previously undescribed in Lytechinus,

could be related to patterns of gonad growth. Finally, latitudinal reproductive variability was examined by comparing observed reproductive phenomena of Lytechinus at Anclote with those described for geographically distant populations.

LITERATURE REVIEW

Intraspecific reproductive variability occurs among both geographically separated populations and proximal populations inhabiting heterogeneous environments. This variability may result from genetic divergence, phenotypic adaptation to dissimilar external stimuli, or both (Sastry, 1975). Locally, exogenous factors interact to produce climates which are compatable to varying degrees with reproductive processes. By comparing the timing, intensity and duration of reproductive cycles among proximal populations, an assessment can be made of the relative suitability of various habitats to an organism's well-being (Moore, 1966).

Reproductive activities can be monitored in a number of ways as outlined by Giese (1959), Boolootian (1966), and Giese and Pearse (1974). The gonad index is a frequently used quantitative method, whereby the amount of gonad material is related to body size, volume or weight. Plotted over time, the gonad index provides information on gonad growth and spawning and the periodicity with which these events occur. The underlying assumption is that the relationship between gonad tissue and body size is linear with respect to all size classes. This assumption may not always hold true, and erroneous conclusions may be drawn when

comparing samples having different size frequency distributions (Argervall and Carlstrom, 1963). This factor may be considerably reduced by utilizing individuals of comparable size. Gonor (1972) has indicated that when properly applied, the gonad index can provide a good evaluation of the compatability of a given habitat to reproductive processes.

Gonad indices reflect only general responses to environmental conditions as measured by changes in the quantity of gonad material present. In many marine invertebrates, requisite nutrient reserves which are utilizied during reproduction are stored in organs other than the gonad (Boolootian, 1966). Thus, the quantity of gonad material present is inversely correlated with the storage organ index (Sastry, 1975). In echinoids, the gonads constitute both a nutrient storage organ and a site for gamete production. Although increases in the gonad index reflect gonad growth, the index does not differentiate between growth related to nutrient accumulation or that associated with gamete production (Boolootian, 1966). Therefore, the index is not definitive in monitoring the formation, development or condition of reproductive products. Consequently, the use of both histological data and gonad indices is highly recommended for the study of reproduction in echinoids (Boolootian, 1966). When a combination of methods has been employed, more explicit definitions of reproductive activities have been obtained (e.g., Pearse, 1969 and 1970).

Both endogenous and exogenous factors are responsible for observed reproductive phenomena. Genetically fixed endogenous factors act to entrain the biological processes associated with reproduction, while exogenous factors serve as cues to entrainment. General reviews of reproductive cycles and animal-environment interactions as they pertain to reproduction have been given by Thorson (1950), Giese (1959), Giese and Pearse (1974), and Sastry (1975).

Several environmental variables, including temperature (Boolootian, 1966: Cochran and Engelmann, 1975; Pearse, 1969 and 1970), salinity (Moore, 1966; Moore and Lopez, 1972), photoperiod (Boolootian, 1966), lunar phase (Moore et al., 1963, Boolootian, 1966), and food availability for developing larvae (Himmelmann, 1975) have been documented to affect the timing and duration of reproduction in various echinoids. These factors may either singularly or collectively serve as cues to activate and/or terminate gametogenic activities and subsequent spawning. They are particularly important integrating agents where synchronous spawning is behaviorly advantageous to the species.

Temperature is perhaps the best studied of the proximate environmental factors affecting reproduction, and is thought to be the primary cue activating endogenous control (Orton, 1920; Thorson, 1950; Gunter, 1957; Giese, 1959; Kinne, 1963 and 1970; Boolootian, 1966; Giese and Pearse, 1974; Sastry, 1975). Critical thermal thresholds must often be satisfied before breeding (spawning) can occur. These thresholds may

occur as maxima, minima or intermediate points along annual oceanic temperature cycles and often account for the intraspecific variation in reproductive timing found among latitudinally separated populations. In general, the effect of temperature on mature adults produces a trend of lengthened spawning with decreasing latitude (Giese and Pearse, 1974; Sastry, 1975).

Food availability, accessibility and type are very important in accounting for local variability in reproductive activities. Differences in gonad size have been noted for various species of urchins taken from different localities (Moore, 1934; Fuji, 1960b; McPherson, 1969; Dix, 1970; Gonor, 1973; Bernard, 1977). These differences are presumably related to food availability, and reduced gonad size may be equated with lower overall spawn production. Lawrence (1975a) has reviewed the relationship between marine flora and sea urchins, and the combined effects of temperature and food availability on reproduction in invertebrates has been treated by Sastry (1975). The acquisition of nutrient reserves and the effect of temperature on nutrient utilization is an intricate relationship which strongly affects reproductive processes.

Reproduction may additionally be influenced by environmental factors related soley to habitat. For example, Oregon populations of the urchin, Strongylocentrotus purpuratus, subjected to heavy wave action, consistently had lesser quantities of gonad material as compared to

populations in more protected waters (Ebert, 1968). The increased energy load channeled to spine repair evidently reduced the capacity for gamete production.

It becomes apparent that reproductive success in sea urchins may be highly dependent on acting environmental conditions. Previous studies of reproduction in Lytechinus have not addressed the problem of environmental heterogeneity. Moore et al. (1963) were aware of this when they suggested that the depicted reproductive cycle of Lytechinus at Miami may have been obscured by the failure to collect specimens at one permanent location. Consequently, the study at Anclote was designed primarily to determine how proximal sub-populations respond reproductively to different habitats. Since nutrient accumulation and gametogenesis may be influenced independently by environmental variables (Gonor, 1973), both aspects of reproduction were monitored. Thus, it was also possible to evaluate the effect of environmental differences on the interrelationship of these processes.

THE STUDY ORGANISM

Lytechinus variegatus has been reported to inhabit a variety of substrates. In southern Florida and the West Indies, this species is most abundant in grass beds (particularly Thalassia), although it may also occur on rocks, in Halimeda beds or on open soft bottoms (Clark, 1933; Moore et al., 1963; Kier and Grant, 1965). In North Carolina, specimens are seldom found in shallow grass beds but are abundant on deeper (2-4 fathoms) sandy shell substrates (Sharp and Gray, 1962).

Moore et al. (1963) reported growth data for Lytechinus at Miami which indicate that it reaches maturity at a test diameter of about 35-40 mm. or between six to eight months after settlement (data extrapolated from figure 7, p. 36). Maximum gonad development is reached at a test diameter of 52-55 mm., after which, the size of the gonads relative to test diameter decreases (Moore et al., 1963). The gonads of Lytechinus are used for human consumption in some areas of the Caribbean (Allain, 1975). Natural populations are believed to live about two or three years attaining a maximum size of nearly 80 mm. (Clark, 1933).

Based on meristic and morphometric data and color, three nominal subspecies of Lytechinus have been described (Serafy,

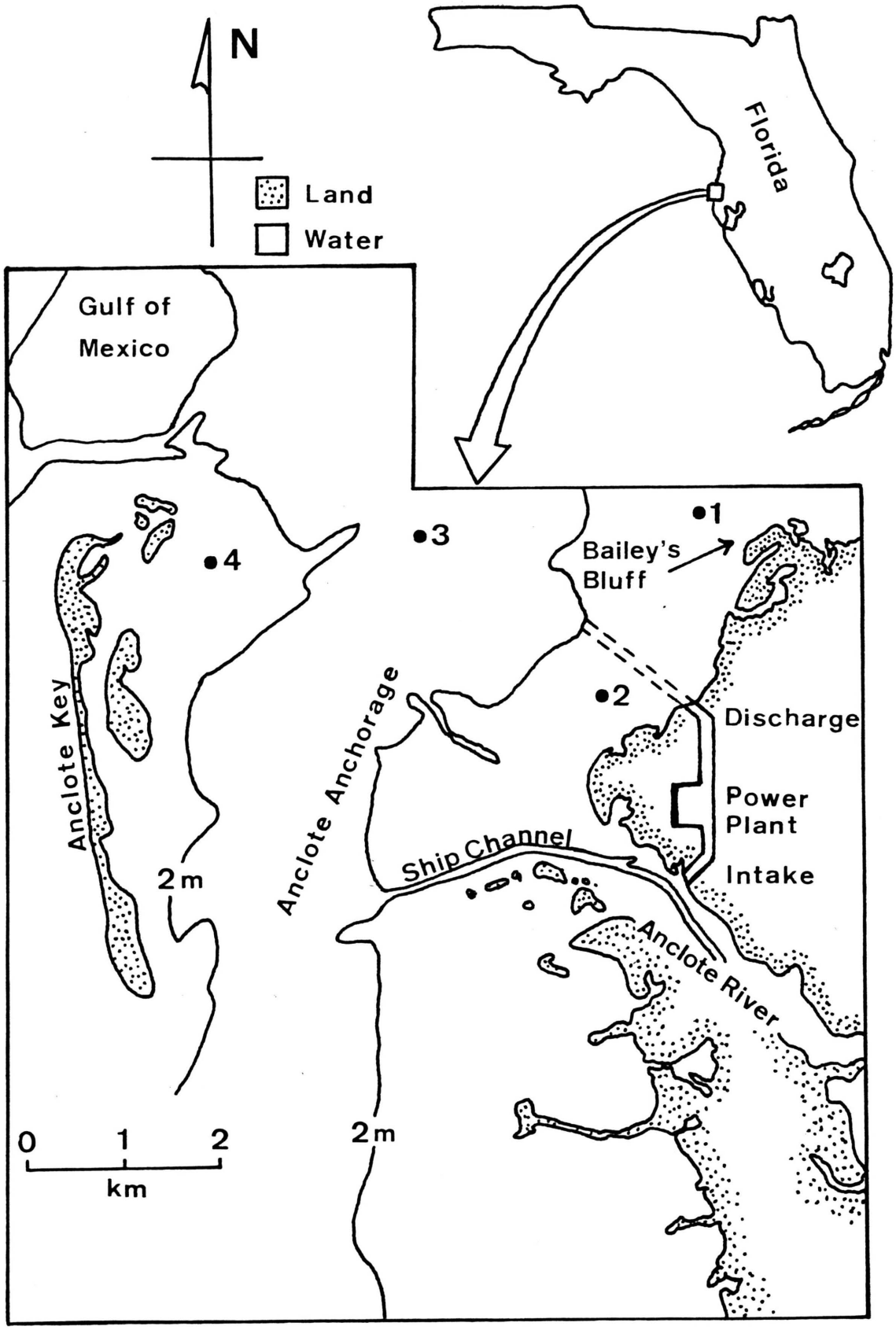
1973). L. variegatus carolinae is found along Florida's west coast, where it is a common component of benthic assemblages.

ENVIRONMENTAL SETTING

Anclote Anchorage is a shallow, semi-protected body of water centrally located on the west coast of Florida near the town of Tarpon Springs (Figure 1). The anchorage and adjoining Anclote River comprise an estuarine system whose geology, chemical and physical hydrology, and biology have been intensively studied during recent years by researchers at the University of South Florida under funding from Florida Power Corporation (Baird et al., 1972, 1973, and 1974; Mayer and Maynard, 1975). The brief summary which follows is drawn from these works with additional information being found in McNulty et al. (1972), and Jones et al. (1973).

Boundaries of the anchorage are delimited by the mainland on the east, the Anclote River/Ship Channel on the south and the barrier islands on the west (Figure 1). The anchorage is open to the north, where it commutes with similar Gulf coastal systems. The average depth of the anchorage is only 1.9 m. (MSL) with depths less than 2 m. extending as muchas 2 km. seaward of the mainland. Shallow flats also occur along the eastern side of the islands. Collectively, these shallow areas support extensive beds of the seagrasses Thalassia testudinum, Syringodium filiforme, and Diplanthera wrightii. Beyond the 2 m. contour, seagrasses become sparse and finally absent in the central portions of the anchorage, eventhough

Figure 1. Anclote Anchorage study site showing location of stations used for evaluating the effect of habitat on the reproductive biology of Lytechinus variegatus.


N
Land
Water
Florida
Gulf of
Mexico
•1
•2
•3
•4
Bailey's
Bluff
Discharge
Power
Plant
Intake
Ship Channel
Anclote Anchorage
Anclote Key
Anclote River
2m
2m
0 1 2
km

greatest depths rarely exceed 5 m. Water clarity is consistently better over grass beds than in deeper regions where bottom deposits are frequently resuspended.

Average monthly water temperatures in the anchorage range from about 12-31°C with coldest temperatures normally being recorded in December and the warmest in August. Except during periods of heavy freshwater runoff, anchorage salinities are relatively high with a range of about 25-35 ppt. Precipitation is usually greatest during the summer when monthly totals may exceed 15 in. (38 cm.). During most of the year, mixing is rapid and thermoclines and haloclines which may exist periodically are quickly dissipated.

Flushing rates for the anchorage have been established from computerized models, and turnover is estimated at 2 to 3 days depending on existing wind velocity, direction and persistence. (Baird,1974) During tidal exchange (flood) the anchorage fills from the west, south-west with river water flowing north into the basin opposite Bailey's Bluff (Figure 1). Following the recent construction of a fossil-fuel power plant(515 MW) just north of the river, much of the river discharge is now drawn through the plant's cooling system. A predicted maximum Δt of 2.8°C may be anticipated during summer months with a Δt of 2.0°C affecting much of the area immediately adjacent to a 1.2 km. discharge canal (FPC, 1977).

Seagrasses cover nearly 40% of the available bottom in the anchorage. Together with numerous species of attached

and drift algae, they provide shelter and a broad trophic base for a diversified fauna. In both vegetated and unvegetated areas are a variety of physical regimes distributed along environmental gradients. The result is a myriad of habitats suitable to a wide spectrum of resident organisms.

METHODS AND MATERIALS

Station Locations

Four stations were selected as sampling sites during the current investigation (Figure 1). The sites chosen met the criteria of having unique habitat characteristics and of harboring sufficient numbers of urchins for repeated collections. Population densities of L. variegatus inhabiting shallow grass beds along the mainland shore were estimated from meter square counts made between July, 1973 and January, 1974 by Blake et al. (1974). Subsequently, other habitats were visually surveyed to locate similar high-density populations. Once the stations had been selected, they were marked with colored floats attached to anchored lines. Fixed landmarks were used to triangulate each position so the station could be accurately relocated in the event the buoys were lost.

Stations 1 and 2 were located near the mainland shore and were characterized by luxuriant beds of mixed seagrasses (Figure 1). Syringodium and Thalassia predominated, while Diplanthera contributed a lesser percent of total cover. Station 1, the more northerly of the two stations, was located in the Bailey's Bluff area. It was considerably removed from the discharge canal of the power plant and thus

was relatively unaffected by thermal additions. Station 2 was located just south of the discharge canal and frequently came under the influence of the heated discharge plume. Although Station 2 was closer to the Anclote River, drainage from numerous canals near Bailey's Bluff coupled with existing circulation patterns in the anchorage, produced relatively uniform salinities at the two stations. Studt (1976) found salinities at these stations to fluctuate seasonally but generally not to differ from each other by more than 2 ppt. Depths at these stations were about 1.5 m. at mean low water.

Station 3 was located in the center of the anchorage near marker 6A in about 4.5 m. of water (Figure 1). Depth at this station precluded the presence of most seagrasses with the exception of an occasional shoot of Diplanthera. Alternate food resources were present in the accumulations of drift algae which settled into depressions on the sea bottom.

Station 4, located on the lee side of Anclote Key in about 2 m. of water, was characterized by alternating patches of Thalassia and bare shelly-sandy substrate. Hydrographic conditions at Stations 3 and 4 were similar and closely approximated Gulf conditions.

Sampling Procedures

Sampling began in late November, 1974 and continued on a monthly basis for one year. Urchins were collected by diving from outboard motor boats except on rare occasions when poor visibility necessitated the use of towed nets. Specimens were collected as encountered on the bottom,

placed in plastic bags and brought on board. They were removed from the bags individually until sufficient numbers within a predetermined size range were obtained. The remaining urchins were returned to the water. When the net was used to collect specimens, individuals were examined carefully, and damaged urchins discarded. All retained specimens were transported to the laboratory in large styrifoam coolers and processing began immediately upon return. Specimens awaiting treatment were maintained in aerated coolers. Because of physical limitations of existing laboratory equipment, not all stations could be sampled on the same day. However, the sampling lag generally did not exceed one week. Sampling dates and numbers of individuals collected at each station are presented in Appendix Tables 15-18. Temperature and salinity data were taken at each station on each trip to the field and at periodic intervals in between.

Gonad Index

Generally, twenty-five urchins per station per sampling date were sacrified for determination of the amount of gonad material present. Prior to dissection, tests of all specimens were measured for diameter and height. Subsequently, each urchin was dissected, the five masses of gonad removed and placed in a pre-weighed aluminum pan, dried to constant weight at 50°C, and then weighed on a Mettler balance to the nearest milligram. The remaining test (including spines) and viscera were treated in the same manner. Sex determinations were made whenever possible by examining gonads for

extruded gametes. If, during extraction, gonads oozed gametes through the severed gonoduct, they were considered ripe.

The gonad index employed during this study is defined as,

$$\frac{\text{dry weight of gonads}}{\text{total dry weight of orgamism}} \quad X \quad 100$$

Thus, the gonad index merely expresses the percentage of total body weight comprised by gonads. Variation in the gonad index resulting from differences in size frequency distributions among individuals from different stations was minimized by using only those urchins with test diameters between 55 and 65 mm. Preliminary underwater surveys indicated that urchins in this size range were readily available. However, on occasion, a few individuals with test diameters slightly below or above this range were used to meet the desired 25 urchin quota.

In order to compare gonad index data derived from this investigation with those obtained by other researchers using different indices, conversion factors were computed. On December 18, 1975, at Station 2, an additional set of 25 urchins was collected and subjected to various treatments including determinations of wet weight and volumetric displacement of intact urchins and removed gonads, dry weight of both gonads and test, and linear measurements of test diameter and height.

Histology

On each sampling trip, gonads from an additional 10 urchins per station were removed and fixed in Dietrichs solution (Yevich and Barszca, 1977). The tissues were later removed from the solution, trimmed, placed in metal cassettes, washed overnight in a running water bath, and then submerged in S-29 (commercial dehydrating agent) for 12 hours. Subsequently, the cassettes were transferred to an Autotechnicon (Technicon Corporation) where they were automatically processed through a series of dehydrating (S-29) and clearing (UC-670) agents and finally immersed in "Paraplast". The cassettes were then placed under pressure in a Paraplast chamber to withdraw air bubbles from tissues. Finally, the tissues were removed from the cassettes and imbedded into Paraplast cubes and a series of sagittal sections 6 um. thick made from each. Once affixed to slides, the sectioned tissues were stained with hematoxylin and eosin according to procedures outlined by Luna (1960).

Gonad material was later examined microscopically and each specimen ranked according to its gametogenic stage of development as described by Fuji (1960a). These stages, summarized in Tables 1 and 2, were used to compute a mean gonad maturity index (Green, 1978). Urchins closest to spawning condition (Stage IV) were assigned the highest rank (5), while those having spent gonads (Stage V) received the lowest (1). The mean for all individuals examined monthly at each station provided a relative estimate of reproductive ripeness. Additionally, each slide containing ovarian tissue

Table 1. Histological stages of gonad development in female sea urchins as characterized by Fuji (1960a).

STAGE	*	NUTRITIVE TISSUE	OOGONIA	OOCYTES	OVA
I (Recovering Spent)	2	Completely fills follicle lumen.	Very numerous in dense clusters along follicle wall.	Relatively small in size and line base of follicle wall.	None present.
II (Growing)	3	Becomes highly globulated as gonad simultaneously increases in size.	Some still present but much less numerous than in previous stage.	Enlarge and become elongate as they begin to push towards center of lumen.	None present.
III (Premature)	4	Restricted primarily to center of lumen.	Few.	Almost completely fill lumen. All sizes present. Many enlarged cells migrate towards center of lumen.	A few present in center of lumen.
IV (Mature)	5	Limited to a thin band around periphery of follicle.	Few.	All sizes present but most restricted to peripheral area of lumen.	Numerous and pack center of lumen.
V (Spent)	1	Limited to periphery but later begins to fill empty lumen.	Increase in number.	Few small cells found along periphery of follicle.	Few unshed ova in center of lumen.

*Gonad Maturity Rank

Table 2. Histological stages of gonad development in male sea urchins as characterized by Fuji (1960a).

STAGE	*	NUTRITIVE TISSUE	SPERMATOGONIA	SPERMATOCYTES	SPERMATOZOA
I (Recovering Spent)	2	Completely fills follicle lumen.	Numerous in small clusters along follicle wall.	Numerous along follicle wall.	None present.
II (Growing)	3	Becomes highly globulated as gonad simultaneously increases in size.	Increase in number along follicle wall.	Increase in number and form widening margin around follicle wall.	None present.
III (Premature)	4	Restricted primarily to center of lumen.	Few.	Increase greatly in number. Margin layer increases in size as many migrate towards center of lumen.	Present in small patches in center of lumen.
IV (Mature)	5	Little present.	Few.	Few limited to periphery of lumen.	Fill nearly entire lumen.
V (Spent)	1	Little present but later begins to fill empty lumen.	Increase in number along follicle wall.	Few along periphery.	Some relict spermatozoa near center.

*Gonad Maturity Rank

was examined and a transect made through a representative portion of the slide. Along the transect, measurements of oocyte and ova diameters were made of the first 50 gametes encountered in which the cross section included the nucleus. The relative quantities of nutrient and gametogenic materials were noted for all specimens, and notes were taken on specimens in which resportion of reproductive products or hermaphroditism occured.

Statistical Treatment

Mean monthly gonad index values were compared using one way analyses of variance (ANOVA). However, during most months, tests for homogeneity of variances and normality of data suggested that the assumptions implicit in the F test could not be met. Numerous transformations were attempted to normalize the data before parametric tests were finally abandoned in favor of non-parametric treatments.

The Kruskal-Wallis (H) and STP (U_s) non-parametric statistics were substituted for ANOVA'S to determine if monthly differences in the gonad index existed among stations (Sokal and Rohlf, 1969). The Kruskal-Wallis test was used to determine if k independent samples had been taken from the same population. Because this statistic does not indicate which samples are different, another statistic, the STP test, was used to ascertain which stations were different during months in which differences occurred. Unlike the Kruskal-Wallis test, the STP statistic requires equal sample size. When necessary, sample size adjustments were made by

eliminating observation numbers higher than n for the sample having the fewest number of observations during that month. Since urchins were taken haphazardly, both in the field and from their retaining coolers in the lab, it is thought that this adjustment did not bias the results. Both the H and U_s parameters are briefly described in Appendices 1 and 2, respectively.

Differences in highest mean monthly gonad index, lowest mean monthly gonad index, and test ratios were also examined with the previous tests. Additionally, paired t-tests were used on histological data to determine if sex ratios at each station departed from uniformity. Urchins collected for gonad index determinations were also used for comparing sex ratios if during a given month at least 10 individuals could be sexed (i.e., ripe). All of the statistical methods were applied at the $P \leq .05$ significance level.

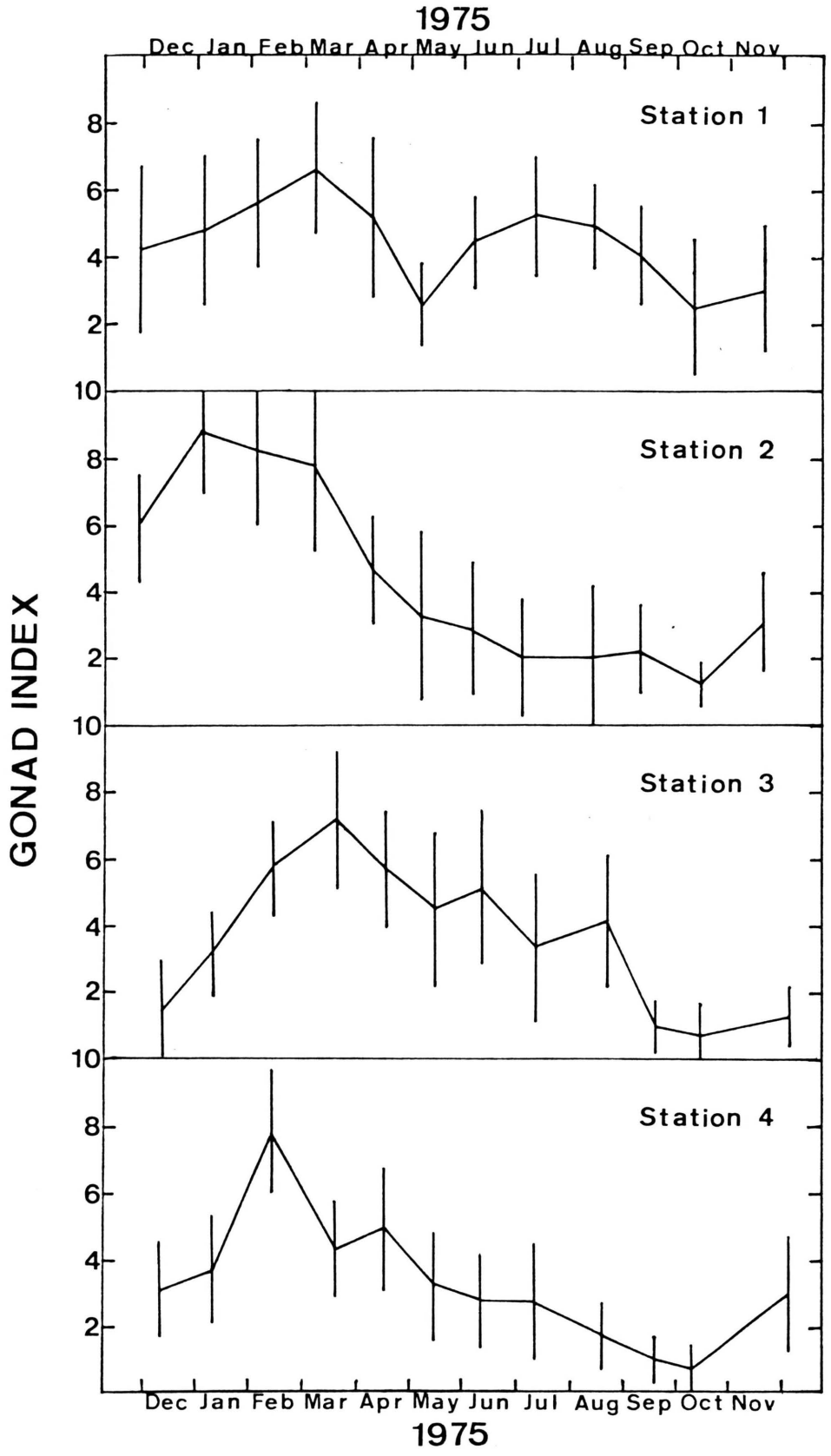
RESULTS

Gonad Index

Mean monthly gonad index values (Appendix Tables 15-18) plotted over the course of a year indicated disparate trends in gonad development among the four stations studied (Figure 2). Initially, the index at all stations increased from early December, 1974, when sampling was initiated, to high levels in February and March, 1975. Subsequently, a substantial decline occurred, suggesting spawning. Except at Station 1 where a bimodal pattern was observed, the gonad index continued to decline throughout spring and summer, reaching minimum values in October. By early December, 1975, gonads once again began to increase in size indicating the initiation of another cycle.

In addition to the March peak, Station 1 data also showed a second but somewhat reduced peak in mid-summer (Figure 2). This second peak was followed by a decline in the index to a minimum level in October and a subsequent increase in December. Although not as pronounced as the bimodal pattern of Station 1, data for Station 3 also showed signs of a second spawning in August (Figure 2). The gonad index at Station 3 declined between March and May, but in contrast to data for Stations 2 and 4, the rate of decline slowed considerably between May and August, the values fluctuating about a

Figure 2. Gonad index curves for samples of Lytechinus variegatus taken monthly at each of four stations. The gonad index = (Dry weight of gonads ÷ Total dry weight of urchin) x 100. Vertical lines represent one standard deviation about the mean.


1975
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
Station 1
Station 2
Station 3
Station 4
GONAD INDEX
2
4
6
8
10
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
1975

constant level. However, between August and September, a marked reduction occurred. Thus, at Stations 1 and 3, there were two apparent spawnings, while at Stations 2 and 4, only one major spawning was evident.

The Kruskal-Wallis (H) non-parametric test (Appendix 1) was applied to mean monthly gonad index values (Appendix Tables 15-18) to determine if apparent differences among stations were statistically significant. The results presented in Table 3 indicate that only during the month of April were there no significant differences ($P \leq .05$) among stations. During that month, most stations were experiencing a decline in the index from high levels the preceeding month (Figure 2). The condition of the urchins collected during April was one in which the gonads during extraction, readily disgorged gametes if cut or agitated. This extremely sensitive condition was universal among urchins from all stations and suggests that spawning which was probably initiated a few weeks earlier was relatively synchronous.

The STP (U_s) non-parametric test (Appendix 2) was applied to the gonad index data for those months in which significant differences had been detected. The results indicate that there was considerable variation in the number of station pairs each month which contained significant differences (Table 3). Furthermore, no pair of stations produced the same results throughout all months studied. Of the 11 months considered, four included differences between three of the six possible station pairs, five had differences between four

pairs, and in September, all but one of the station pairs (3-4) was significantly different. In May, as gonad index values were decreasing coincident with spawning, only one pair of stations (1-3) was significantly different. Fewest monthly differences (5) were between Stations 2 and 4, while Stations 2 and 3 had the largest number of monthly differences (8).

In addition to the disparities in the mean monthly gonad index among stations, differences also existed in the timing and absolute values of peak gonad development (Figure 2). At Station 2, the largest mean monthly index value was in January, at Station 4 in February, and at Stations 1 and 3 in March. The Kruskal-Wallis test indicated that the highest attained mean monthly gonad index differed significantly ($P \leq .05$) among stations (Table 4). The STP test revealed that Station 2 which had the highest mean index value (8.8) computed during the study was significantly greater than both Stations 1 and 3 (Table 4). No other combination of paired stations was significantly different.

The lowest mean monthly index value at all stations occurred in October. Again there weresignificant differences ($P \leq .05$) among stations (Table 4). Station 1, which had the largest value that month, was different from both Stations 3 and 4. Station 2 was also different from Stations 3 and 4, while station pairs 1-2 and 3-4 were not significantly different. During the entire course of study, the highest and lowest index values calculated for an individual urchin were 13.3 (March, Station 2) and 0.1 (October, Station 3), respectively.

Table 3. Results of Kruskal-Wallis (H) and STP (U_s) non-parametric tests applied to mean monthly gonad index values. Tabulated H values are taken from Rohlf and Sokal (1969), Table R. N represents the number of individuals per station used for computing U_s values. See Appendices 1 and 2 for complete computational methods.

Month	H	Tabulated H	N	U_s For Paired Stations 1-2	1-3	1-4	2-3	2-4	3-4	Critical U_s
December, 1974	48.2*	7.82	25	444	528*	396	609*	567*	508*	444.9
January, 1975	55.1*	7.82	25	566*	473*	414	620*	612*	380	444.9
February, 1975	28.2*	7.82	24	468*	305	460*	470*	324	469*	415.1
March, 1975	31.3*	7.82	25	389	344	519*	342	562*	539*	444.9
April, 1975	4.8	7.82		Not Computed						
May, 1975	10.9*	7.82	25	335	464*	393	427	357	422	444.9
June, 1975	25.3*	7.82	25	488*	381	509*	475*	325	503*	444.9
July, 1975	31.4*	7.82	25	561*	484*	540*	432	399	358	444.9
August, 1975	53.1*	7.82	23	481*	355	514*	448*	281	471*	381.4
September, 1975	53.9*	7.82	21	364*	430*	434*	337*	343*	223	322.6
October, 1975	34.4*	7.82	21	294	372*	366*	345*	331*	228	322.6
November, 1975	27.8*	7.82	25	342	527*	317	549*	337	519*	444.9

* Significant at $P \leq .05$

Table 4. Results of Kruskal-Wallis (H) and STP (U_s) non-parametric tests applied to highest and lowest mean monthly gonad index values. Tabulated H values are taken from Rohlf and Sokal (1969), Table R. N represents the number of individuals per station used for computing U_s values. See Appendices 1 and 2 for complete computational methods.

KRUSKAL-WALLIS TEST

Station	Highest Mean Monthly Goand Index	Date	Lowest Mean Monthly Gonad Index	Date
1	6.68	3/6/75	2.51	10/9/75
2	8.79	1/3/75	1.25	10/14/75
3	7.15	3/13/75	0.76	10/14/75
4	7.83	2/13/75	0.74	10/9/75
H	15.93*		34.40*	
Tabulated H	7.82		7.82	

STP

Highest Mean Monthly Gonad Index							Lowest Mean Monthly Gonad Index						
1-2	1-3	1-4	2-3	2-4	3-4	N	1-2	1-3	1-4	2-3	2-4	3-4	N
490*	325	443	460.5*	338.5	409	25	294	372*	360*	345*	331*	228	21
Critical U_s = 444.9							Critical U_s = 322.6						

* Significant at $P \leq .05$

A conversion factor was computed to allow comparison of gonad index curve amplitudes among Bermuda, Miami, and Anclote populations of Lytechinus. Gonad indices calculated from dry weight measurements averaged 7.2 times higher than gonad indices calculated from volumetric measurements (Table 5). At Miami, highest gonad indices calculated for each of 10 years, ranged from 0.8 to 2.0, and averaged 1.5 (Moore and Lopez, 1972). At Bermuda, the single year gonad index curve reached a high of 2.0 (Moore et al., 1963). During the current study, the highest mean monthly value for any station equated to 1.2 on the volumetric scale, and the highest values for all stations combined averaged only 1.1. Thus, for the 1975 reproductive cycle of Lytechinus at Anclote, gonad production was considerably less than the average peak production at Miami or Bermuda.

The capacity for dramatic changes in the relative amount of gonad material present is apparent from the gonad index curves (Figure 2). At Station 1, for example, the gonad index experienced a 60% decline during the two month period in which the first spawning occurred (March-May). At Station 3, between December, 1974 and the peak in March, 1975, there was an increase of 380% in the gonad index. Similarly, at Station 4, an increase of 150% occurred between December, 1974 and the peak in February, 1975.

The percent monthly change in relative gonad size was computed for each station and comparisons among stations made possible by calculating the average daily change (Table 6).

Table 5. Comparison of gonad indices derived from dry weight and volumetric measurements

Specimen	Gonad Volume (ml)	Gonad Dry Weight (g)	Gonad Wet Weight (g)	Test Volume (ml)	Test Dry Weight (g)
1	7	1.12	6.57	100	24.3
2	6	1.11	7.21	100	25.8
3	4	0.78	4.54	120	28.9
4	4	0.56	3.99	80	19.0
5	8	1.88	9.20	100	28.4
6	9	1.26	9.00	100	21.3
7	5	0.90	5.55	100	22.3
8	16	1.35	19.07	130	28.5
9	7	1.01	6.97	90	21.4
10	6	0.98	5.34	120	23.6
11	6	1.28	6.56	130	33.2
12	4	0.74	3.81	75	18.1
13	4	1.13	5.32	100	26.1
14	4	0.93	4.96	100	35.1
15	2	0.51	2.65	85	23.3
16	5	0.63	3.63	120	29.1
17	4	0.75	3.83	130	31.1
18	8	1.49	8.53	95	21.5
19	5	0.79	4.73	85	20.7
20	10	2.15	10.99	90	23.7
21	5	0.79	5.42	100	23.8
22	8	1.41	9.18	85	22.0
23	6	0.96	5.52	80	20.2
24	3	0.66	3.39	80	19.3
25	3	0.70	3.20	75	19.6
$\bar{X}$	5.96	1.04	6.37	98.8	24.4
S	2.89	0.40	3.43	17.3	4.6

Table 5 (cont'd).

Specimen	Test Wet Weight (g)	Test Diameter (mm)	GI-1 *	GI-2 **	GI-1 / GI-2
1	118.4	60	4.41	0.70	6.29
2	109.1	59	4.14	0.60	6.90
3	129.8	63	2.63	0.33	7.98
4	93.4	55	2.86	0.50	5.71
5	117.3	61	6.21	0.80	7.76
6	108.8	59	5.56	0.90	6.18
7	107.7	59	3.90	0.50	7.80
8	149.5	65	4.53	1.23	3.68
9	105.7	56	4.49	0.78	5.76
10	125.2	62	3.98	0.50	7.96
11	149.8	64	3.70	0.46	8.04
12	84.6	54	3.91	0.53	7.38
13	117.9	62	4.14	0.40	10.36
14	110.8	61	3.57	0.40	8.93
15	102.1	57	2.15	0.24	8.94
16	129.7	61	2.11	0.42	5.04
17	147.6	65	2.36	0.31	7.61
18	107.4	60	6.50	0.84	7.74
19	100.6	59	3.65	0.59	6.18
20	104.2	59	8.32	1.11	7.49
21	111.5	63	3.19	0.50	6.39
22	101.2	58	6.03	0.94	6.41
23	93.9	57	4.52	0.75	6.03
24	94.3	57	3.29	0.37	8.89
25	84.9	54	3.46	0.40	8.64
$\bar{X}$	112.1	59.6	4.14	0.60	7.20
S	18.2	3.2	1.47	0.26	1.46

* $\dfrac{\text{Gonad Dry Weight}}{\text{Gonad Dry Weight + Test Dry Weight}} \times 100$

** $\dfrac{\text{Gonad Volume}}{\text{Test Volume}} \times 10$

Table 6. Percent monthly and daily changes in gonad weight of urchins taken from four stations in the Anclote Anchorage

Percent change from pervious month					Percent daily change				
	Station					Station			
Month	1	2	3	4	Month	1	2	3	4
January	13	48	111	18	January	.4	1.4	3.8	.6
February	18	-6	82	110	February	.6	-.2	2.3	3.1
March	18	-5	25	-44	March	.6	-.2	.8	-1.3
April	-22	-40	-21	13	April	-.7	-1.2	-.7	.4
May	-49	-30	-21	-35	May	-1.7	-1.1	-.7	-1.3
June	71	-13	15	-13	June	2.4	-.4	.6	-.5
July	17	-29	-35	-1	July	.6	-1.1	-1.2	0
August	-07	0	24	-36	August	-.2	0	.6	-.9
September	-18	11	-75	-45	September	-.6	.4	-2.8	-1.5
October	-38	-45	-27	-24	October	-1.3	-1.3	-1.0	-1.1
November	23	150	67	299	November	.6	4.2	1.3	5.4

At Station 1, average daily increases during gonad growth phases were below 1%/day except in June, when the second increase in the gonad index began. At all other stations, average daily gonad growth often exceeded 1%/day during initial gonad growth following spawning. The highest average daily increase was computed for Station 4 (5.4%/day) during November when gonads were increasing in size coincident to the initiation of another annual cycle. The largest average daily decrease occurred at Station 3 (2.8%/day) during the final spawnout between August and September.

The disparity in station trends depicted by gonad index data may be briefly summarized as follows: During the winter, gonads increased in size at various rates among the stations and attained maximum size during different months in late winter and early spring. Rapid declines in index values during spring occurred at all stations suggesting a relatively synchronous initial spawning period throughout the Anchorage. In March, gonad index values at Stations 1, 2, and 3 were at high levels prior to spawning and these stations did not differ significantly from one another. Station 4 data, which showed a peak in the index in February, had a significantly lower index value during March, indicating that spawning had commenced earlier than at other stations. During April, none of the stations differed significantly and during May, as spawning continued, only one pair of stations had significantly different gonad index values. Throughout the summer and early fall, index values

fluctuated in various manners, and differences among stations varied eratically. At Station 1, gonads began a second growth phase in early summer producing a bimodal reproductive pattern. Urchins at Station 3 maintained relatively high gonad index values throughout the summer with a marked reduction in September. Secondary summer spawnings at Stations 2 and 4 were not evident from gonad index curves as index values at those stations declined steadily throughout spring and summer. Lowest values at all stations occurred in October indicating apparent completion of the reproductive cycle.

If gonad quantity is a good indicator of spawn production, the area under the gonad index curves may provide the best estimate of quantities of gametes obtainable at each station. The areas under the curves were estimated by constructing histograms between sampling periods such that the height of each histogram was equal to the mean of the index values between months, and the width was equivalent to the time in days between sampling periods. This method produced areas for 11 histograms at each station. Total areas under the curves ranged from 1,068 units at Station 1 to 1,167 units at Station 4. Stations 2 and 3 had intermediate values of 1,534 and 1,324 units, respectively. Although the difference between high and low values seems appreciable, the Kruskal-Wallis statistic applied to the data detected no significant differences. ($P \leq .05$)

Histology

Gametogenic stages and activities observed in the specimens of Lytechinus variegatus collected at Anclote are briefly described below.

Stage I

The follicles of Stage I urchins were large and most were completely filled with nutritive tissue. Some still contained a small vacated space in the central lumen, but the few remaining ova and spermatozoa were being lysed by nutritive phagocytes (Chatlynne, 1969) which encroached and subsequently filled the empty space. The nutritive tissue contained conspicuous globules characteristic of this stage of development. At the base of the germinal layer, ovarian follicles contained oogonia which were usually in clusters and primary oocytes aligned on an axis parallel to the follicle wall. Oogonia were generally about 5 um. in diameter, and oocytes varied from about 10 to 30 um. in length. Testicular follicles contained numerous spermatogonia and spermatocytes along the follicle wall. This thin band was clearly discernible from lighter staining nutritive tissue.

Stage II

Stage II urchins had central lumina completely filled with highly globulated and sometimes vacuolated nutritive tissue. Primary oocytes which formed nearly continuous bands around the ovarian follicles enlarged and proliferated and had become more elongate as they began to protrude inwards

toward the center of the follicle. The smaller primary oocytes which varied in shape had diameters of 20 to 30 um., while the more advanced germinal cells, whose long axes were now perpendicular to the cell wall, were up to 60 um. in length. Oocytes during this stage were characterized by a round germinal vesicle in the central region of the cell which measured 20 to 30 um. in diameter. In the testes, spermatogenesis had proceeded and the follicle was margined with numerous primary and secondary spermatocytes. Neither ovarian nor testicular tissue contained mature gametes in the follicles.

Stage III

Stage III urchins were in a state of advanced gametogenesis, mature gametes being present in small numbers in the center of some of the follicles. In the ovaries, advanced oocytes had detached from the follicle walls, and as they migrated towards the center of the follicle, they simultaneously began a cytoplasmic growth phase. Breakdown of the now enlarged nucleus was observed as the oocytes neared the center and meiosis and vitellogenesis began. Although ovarian tissue possessed oocytes of all sizes, the majority of space was filled with elongate advanced oocytes which measured about 80-150 um. by 40-80 um. In the testes, secondary spermatocytes were migrating towards the center of the follicle and dividing to form spermatids.

Stage IV

Reduction division neared completion in Stage IV urchins, and mature gametes filled the lumina of the follicles. In females, ova packed most of the lumina except in some peripheral accini which contained oocytes of all sizes similar to Stage III gonads. In most follicle lumina, the nutritive phagocite layer was restricted to a thin band along the periphery of the follicle containing a few small primary oocytes. During this stage, there were few globules remaining in the nutritive tissue. Mature ova, which measured about 100 um. in length, did not differ substantially in size from advanced Stage III oocytes. In the male follicle, the spermatids elongated and the resulting spermatozoa filled most of the available space. Spermatogenesis continued along the periphery of the follicle although at a reduced rate.

Stage V

The follicles of Stage V urchins were empty except for a thin peripheral band of nutritive tissue containing immature gametes and a few unshed mature gametes in the center. The relict gametes were apparently phagocytized by nutritive cells which subsequently filled the open spaces. Oogonia and spermatogonia progressively increased in number as the follicle began to return to the Stage I condition.

Although the timing of gametogenic activities differed slightly among stations, the progression of events was identical. The number of urchins belonging to each stage of

development is presented for all stations in Tables 7-10, and mean monthly ranks of gonad maturity are plotted against the gonad index for all stations in Figure 3. Two periods of advanced gametogenic activity are apparent from the curves. The first occurred in spring and closely corresponds to the time of highest mean monthly gonad index values. The second peak came in late summer and projects a period of increased gametogenic activity not evident from gonad index curves at most stations. Spring peaks were much more pronounced than late summer peaks and reflect the synchronous condition of urchins during the early part of the year. As spawning and recovery took place at diffential rates among individuals within each population, mean monthly gonad maturity values declined.

Gonad maturity curves suggest that the major spawning of the year took place between March and April at Stations 1 and 2 and between April and May at Stations 3 and 4 (Figure 3). Station 4 may have experienced a partial spawning between February and March as well. The final spawnout of the cycles came between September and October at all stations except Station 3, where it appears to have commenced in August. The presence of some mature (Stage IV) and some spent (Stage V) urchins during most months (Tables 7-10) suggests that spawning may be continuous over much of the year with a certain percentage of urchins in each of the different stages of gonadal development.

As might be expected, the curves generated from mean

Table 7. Histological information obtained from 10 urchins collected monthly at Station 1. See Tables 1 and 2 for determination of state of development and corresponding ranks for female and male gonads, respectively.

Date	Stage of Development Male I	II	III	IV	V	Female I	II	III	IV	V	Number of Males	Number of Females
11/29/74	5				1	3	1				6	4
1/3/75	3	1	1				2	3			5	5
2/2/75	1	1	1	1		2		1	3		4	6
3/6/75				2					8		2	8
4/8/75		2	2	2				1	3		6	4
5/6/75	4		1		1		1		3		6	4
6/5/75	4					2	2	1	1		4	6
7/2/75	2					4	2	2			2	8
8/12/75	4	2				1	1	2			6	4
9/9/75	2	1	1	2				1	2	1	6	4
10/9/75	4									6	4	6
11/19/75	2	1			1		2			3	4	5

Table 7 (cont'd.)

Date	Sex Ratio (M/F)	Mean Rank Males	Mean Rank Females	Mean Rank Both Sexes	Mean Oocyte Diameter
11/29/74	1.50	1.83	2.25	2.00	34.6
1/3/75	1.00	2.60	3.60	3.10	54.3
2/2/75	0.67	3.50	3.83	3.70	56.2
3/6/75	0.25	5.00	5.00	5.00	54.9
4/8/75	1.50	4.00	4.75	4.30	56.8
5/6/75	1.50	2.17	4.50	3.10	43.1
6/5/75	0.67	2.00	3.17	2.70	40.2
7/2/75	0.25	2.00	2.75	2.60	31.9
8/13/75	1.50	2.33	3.25	2.70	36.8
9/9/75	1.50	3.50	4.00	3.70	51.3
10/9/75	0.67	2.00	1.00	1.40	42.3
11/19/75	0.80	2.00	1.80	1.80*	36.8

*Includes stage V urchins which could not be sexed

Table 8. Histological information obtained from 10 urchins collected monthly at Station 2. See Tables 1 and 2 for determination of stage of development and corresponding ranks for female and male gonads, respectively.

Date	Stage of Development: Male I	II	III	IV	V	Female I	II	III	IV	V	Number of Males	Number of Females
11/29/74	6				1	1	2				7	3
1/3/75		3				6	1				3	7
2/3/75	4	5						1			9	1
3/6/75				5					5		5	5
4/8/75	2		4	1				2	1		7	3
5/6/75	3	1	1				1		2	2	5	5
6/5/75	6					2	2				6	4
7/2/75	3				1					5	4	5
8/12/75					2	1				3	2	4
9/9/75	2		1			1		1	1	4	3	7
10/14/75	3				2		2			3	5	5
11/9/75	3	3				3				1	6	4

Table 8 (cont'd.)

Date	Sex Ratio (M/F)	Mean Rank Males	Mean Rank Females	Mean Rank Both Sexes	Mean Oocyte Diameter
11/29/74	2.33	1.86	2.67	2.10	31.1
1/3/75	0.43	2.00	2.14	2.40	28.1
2/3/75	9.00	2.56	4.00	2.70	53.6
3/6/75	1.00	5.00	5.00	5.00	57.6
4/8/75	2.33	4.14	4.33	4.20	55.3
5/6/75	1.00	2.60	3.00	2.80	46.8
6/5/75	1.50	2.00	2.50	2.20	26.3
7/2/75	0.80	1.75	1.00	1.30*	15.2
8/12/75	0.50	1.00	1.25	1.10*	29.7**
9/9/75	0.43	2.67	2.14	2.30	47.3**
10/14/75	1.00	1.60	1.80	1.70	36.4**
11/19/75	1.50	2.50	1.75	2.20	28.4

* Includes Stage V urchins which could not be sexed
** Some females in Stage V condition had insufficient number of oocytes to be measured.

Table 9. Histological information obtained from 10 urchins collected monthly at Station 3. See Tables 1 and 2 for determination of stage of development and corresponding ranks for female and male gonads, respectively.

	Stage of Development											
	Male					Female					Number of	Number of
Date	I	II	III	IV	V	I	II	III	IV	V	Males	Females
12/11/74	2				2	2		2		2	4	6
1/9/75	4	1	1			1	2		1		6	4
2/13/75			4		2		1		1		8	2
3/18/75	1	4	3					2			8	2
4/16/75			2	3				1	4		5	5
5/14/75				2	4	3				1	6	4
6/10/75	6					2	2				6	4
7/10/75	7				1	1				1	8	2
8/21/75	2				3		1	2	1		5	4
9/17/75				1	5	2				2	6	4
10/14/75	3				3					4	6	4
12/3/75	3	1			2	1				3	6	4

Table 9 (cont'd.)

Date	Sex Ratio (M/F)	Mean Rank Males	Mean Rank Females	Mean Rank Both Sexes	Mean Oocyte Diameter
12/11/74	0.67	1.50	2.33	2.00	30.4
1/9/75	1.50	2.50	3.25	2.80	40.8
2/13/75	4.00	2.25	4.00	2.60	48.6
3/18/75	4.00	3.25	4.00	3.80	54.6
4/16/75	1.00	4.60	4.80	4.70	54.3
5/14/75	1.50	2.33	1.75	2.10	25.2
6/10/75	1.50	2.00	2.50	2.20	24.7
7/10/75	4.00	1.88	1.50	1.80	17.4
8/21/75	1.25	1.80	4.00	2.50	35.6
9/17/75	1.50	1.50	1.50	1.50	27.7
10/14/75	1.50	1.50	1.00	1.30	23.0**
12/3/75	1.50	1.83	1.25	1.60	13.6

**Some females in Stage V condition had insufficient numbers of oocytes to be measured.

Table 10. Histological information obtained from 10 urchins collected monthly at Station 4. See Tables 1 and 2 for determination of stage of development and corresponding ranks for female and male gonads, respectively.

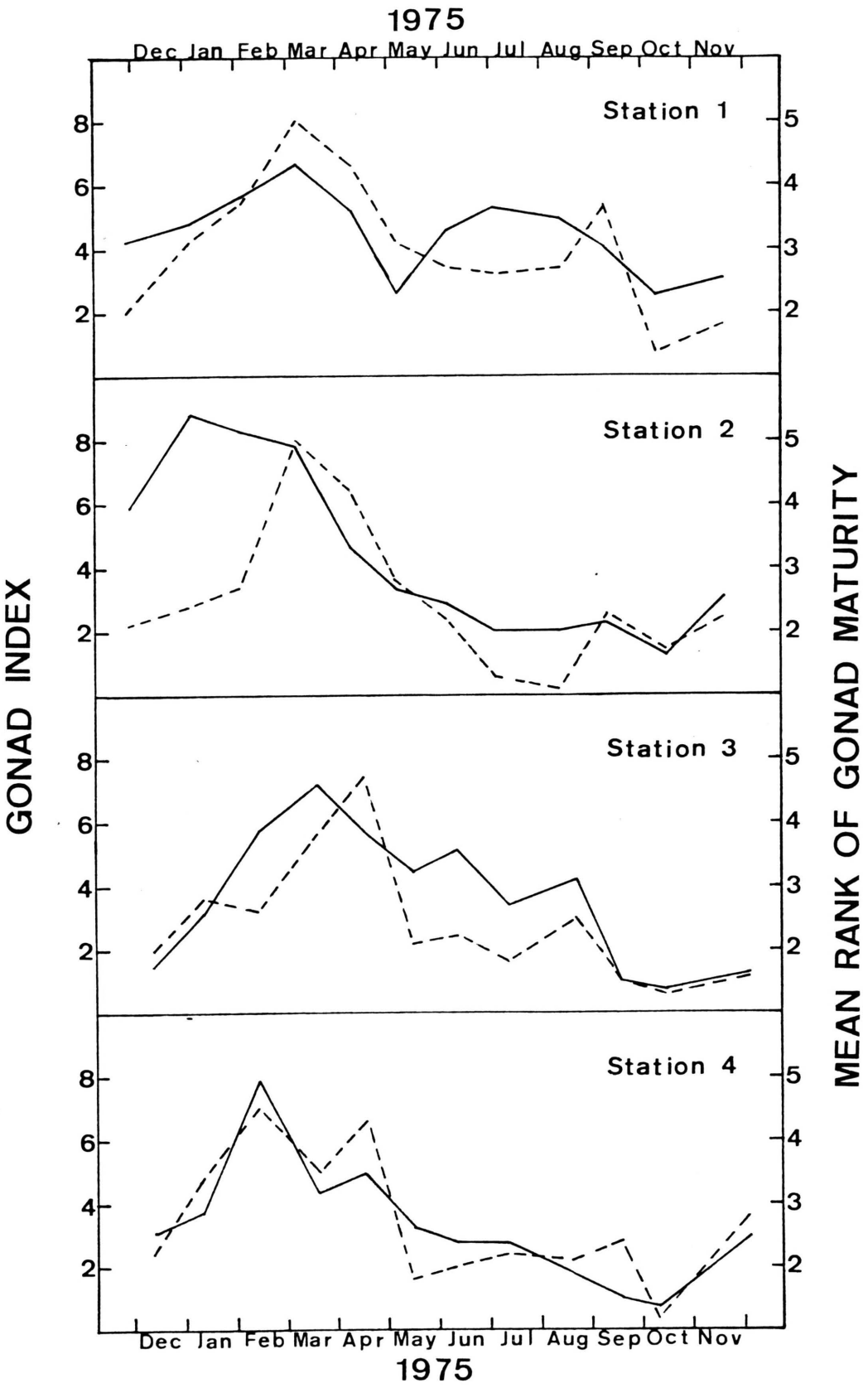
Date	Stage of Development										Number of Males	Number of Females
	Male					Female						
	I	II	III	IV	V	I	II	III	IV	V		
12/11/75	2	2			1	1	1	1		1	5	4
1/9/75		5	3			1			1		8	2
2/13/75			4	1				1	4		5	5
3/18/75	1		2		1			2	3	1	4	6
4/16/75			4					3	3		4	6
5/14/75			1	1	5	1				2	7	3
6/10/75	7					3					7	3
7/10/75	4	2				4					6	4
8/18/75	2	1			2	3	2				5	5
9/17/75	1		2		2	1			2	2	5	5
10/9/75	1				4	1				4	5	5
12/3/75	2	3					5				5	5

Table 10 (cont'd).

Date	Sex Ratio (M/F)	Mean Rank Males	Mean Rank Females	Mean Rank Both Sexes	Mean Oocyte Diameter
12/11/75	1.25	2.20	2.50	2.20*	30.4
1/9/75	4.00	3.37	3.50	3.40	36.3
2/13/75	1.00	4.20	4.80	4.50	50.1
3/18/75	0.67	2.75	4.00	3.50	50.3
4/16/75	0.67	4.00	4.50	4.30	50.1
5/14/75	2.33	2.00	1.33	1.80	22.3
6/10/75	2.33	2.00	2.00	2.00	22.2
7/10/75	1.50	2.33	2.00	2.20	14.5
8/18/75	1.00	1.80	2.40	2.10	22.1
9/17/75	1.00	2.40	2.40	2.40	32.9
10/9/75	1.00	1.20	1.20	1.20	22.6**
12/3/75	1.00	2.60	3.00	2.80	27.2

* Includes Stage V urchins which could not be sexed
** Some females in Stage V condition had insufficient number of oocytes to be measured

Figure 3. Relationship between gonad index (solid line) and mean rank of gonad maturity (dotted line) for urchins collected monthly at each of four stations. Gonad maturity ranks are for both sexes combined.


1975
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
Station 1
Station 2
Station 3
Station 4
GONAD INDEX
MEAN RANK OF GONAD MATURITY
8
6
4
2
5
4
3
2
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
1975

oocyte diameters closely paralleled those of the gonad maturity curves (Figure 4). However, mean oocyte diameter curves reached high points earlier than gonad maturity curves. This can be accounted for by the nearly equal sizes of advanced oocytes (Stage III) and mature ova (Stage IV). Thus, at Station 1, mean oocyte diameters approached maximum values in January due to the presence of several Stage III females (Figure 4). It remained at this high level through April, a month after spawning had begun, because of the continued presence of Stage III females in the samples. Mean oocyte diameters plotted against gonad index curves for each station produced trends identical to those generated from mean gonad maturity data and substantiate major spawnings in spring and late summer (Figure 5).

Since mean oocyte diameter is related only to the female contribution to the gonad maturity curves, a mean female gonad maturity curve was plotted to determine how it varied from the overall mean rank curves for both sexes combined (Figure 4). It is evident that the two are in close agreement except that the mean female curve is slightly higher. All three histological gauges were closely aligned, and all indicate the presence of two peaks of gametogenic activity.

One other index of reproductive activity was obtained by determining the percentage of ripe individuals contained in each month's collection (Table 11). Figure 6 plots the percentage of ripe individuals at each station against gonad index curves. Again two periods of spawning are evident.

Figure 4. Mean rank of gonad maturity for females (dotted line) and both sexes combined (solid line) and mean oocyte diameter (- · -) for urchins collected monthly at each of four stations.

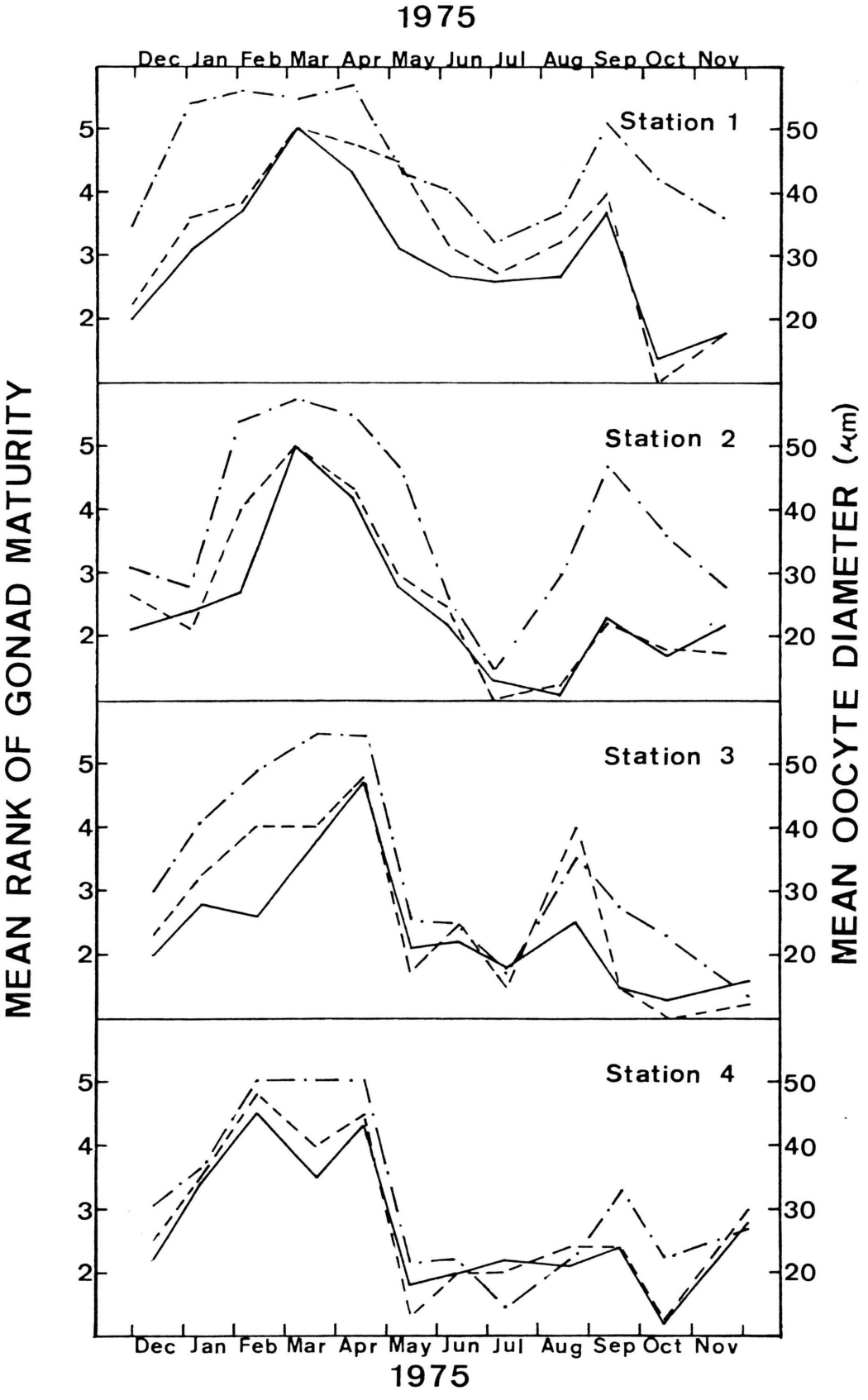

1975
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
Station 1
Station 2
Station 3
Station 4
MEAN RANK OF GONAD MATURITY
MEAN OOCYTE DIAMETER (μm)
5
4
3
2
50
40
30
20
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
1975

Figure 5. Relationship between gonad index (solid line) and mean oocyte diameter (dotted line) for urchins collected monthly at each of four stations.

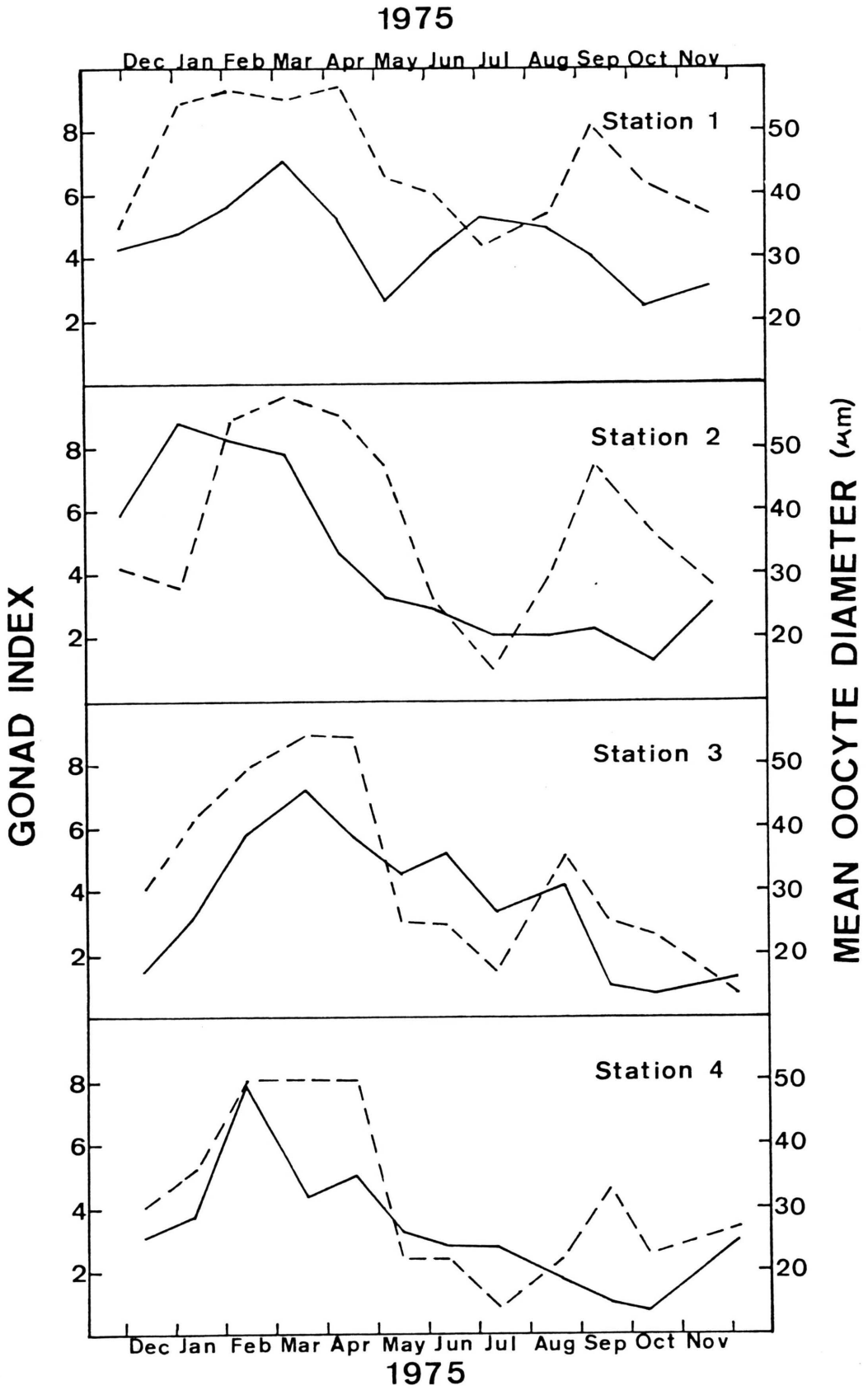

1975
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
Station 1
Station 2
Station 3
Station 4
GONAD INDEX
MEAN OOCYTE DIAMETER (μm)
8
6
4
2
50
40
30
20
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
1975

Figure 6. Relationship between gonad index (solid line) and percent of individuals oozing ripe gametes upon sectioning (dotted line).

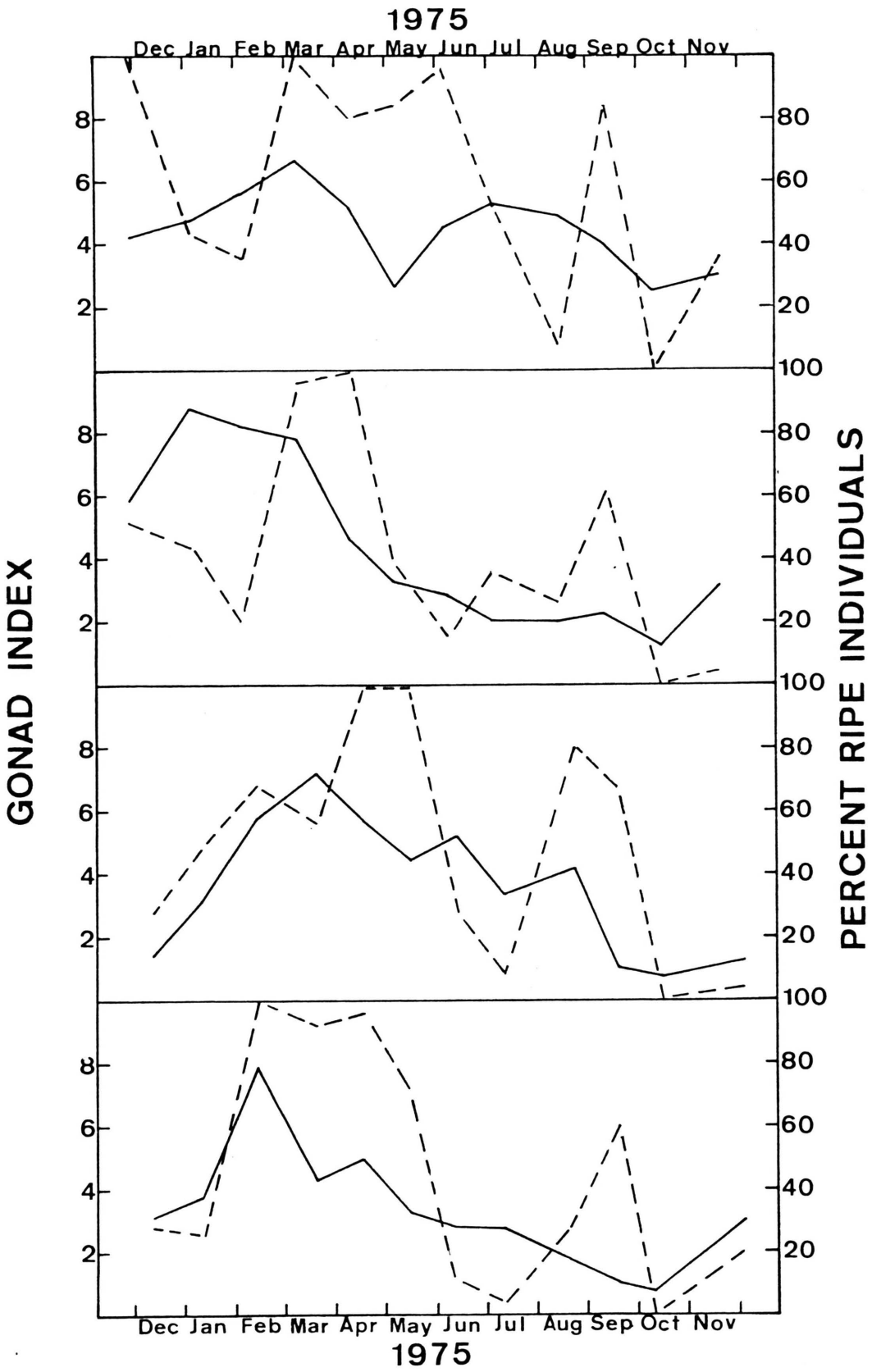

1975
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
GONAD INDEX
PERCENT RIPE INDIVIDUALS
2
4
6
8
20
40
60
80
100
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
1975

Table 11. Percent of ripe individuals contained in monthly collections from four stations. Ripeness criteria based on the shedding of gametes when gonads sectioned. N represents the number of individuals examined.

	Station							
	1		2		3		4	
Month	N	%	N	%	N	%	N	%
December	25	100	25	52	25	28	25	28
January	25	44	25	44	25	48	25	26
February	25	36	25	20	25	88	25	100
March	25	100	25	96	25	76	25	92
April	25	80	25	100	25	100	25	96
May	25	84	25	40	25	100	25	72
June	25	96	25	16	25	28	25	12
July	25	52	25	36	25	8	25	4
August	25	8	23	26	25	80	25	28
September	25	84	23	61	21	67	25	60
October	25	0	21	0	24	0	25	0
November	25	36	25	4	25	4	25	20

At Station 1, large numbers of ripe individuals were present from March through June, while at Station 2, a decline in the relative number of ripe individuals occurred in May. At Station 3, ripe individuals were numerous between February and May, and at Station 4 between February and April. Another peak of ripe individuals occurred in September (also August at Station 3) at all stations. As with other histological indices, the initial spring peak was higher than the late summer peak indicating an erosion of synchrony in gametogenic activity towards the end of the annual cycle.

Sex Ratios

The mean number of male and female urchins collected each month was compared for each station to determine if sex ratios departed significantly from uniformity. Paired t-tests applied to histological samples indicated that only at Station 3 did the sex ratios differ significantly ($P \leq .05$) from 1, with males predominating (Table 12). However, when gonad index collections were used to make comparisons between sexes, no significant differences were found at any of the stations. Although monthly means were substantially larger for gonad index than for histological collections (due to larger sample sizes), there were fewer degrees of freedom for statistical comparisons. This was because only during certain months was sexing possible by gross observation (i.e., shedding gametes).

Morphology

Mean test ratios compared with the Kruskal-Wallis (H)

Table 12. Results of t-tests performed on mean sex ratios (males:females) of urchins collected monthly for gonad index and histological purposes at each of four stations. (H=histological collection and GI=gonad index collection)

	1		2		3		4	
	H	GI	H	GI	H	GI	H	GI
Males Total No.	55	66	62	35	74	58	66	57
Mean No./Month	4.58	11.0	5.17	8.7	6.17	9.7	5.50	11.4
Standard Deviation	1.5	3.0	2.0	5.6	1.3	1.5	1.2	2.3
Females Total No.	64	58	53	42	45	67	53	47
Mean No./Month	5.33	9.67	4.42	10.5	3.75	11.2	4.42	9.4
Standard Deviation	1.5	2.2	1.7	7.0	1.2	4.3	1.2	1.7
Mean Sex Ratio	0.86	1.17	1.17	1.37	1.65	1.01	1.24	1.22
t	-0.87	1.08	0.74	-0.33	*3.39	-0.76	1.52	1.75
df	11	5	11	3	11	5	11	4
Tabulated t	2.20	2.6	2.20	3.2	2.20	2.6	2.20	2.8

*Significant at $P \leq .05$

non-parametric statistic were found to be significantly different (Table 13). When the STP test was applied, it was found that Stations 1 and 2, located in similar habitats, did not differ from one another, but every other of the six possible station pairs was significantly different. The ratios describe the general shape of the test, and it is apparent that Station 3 urchins had the lowest profile (i.e. flattest test), while urchins at Stations 1 and 2 had the highest (most rounded).

Temperature and Salinity

Coldest water temperatures during the study period were recorded in December, 1974, while the warmest temperatures were observed in late July and August, 1975. Bottom water temperatures ranged from a low of 12.5°C at Station 4 (December, 1974) to a high of 33.5°C at Station 2 (August, 1975). All stations showed the same trend of gradually increasing temperatures between December and May with relatively high levels maintained between May and September (Appendix Table 19). Rapid decreases were observed between October and November, as the first winter cold fronts began passing through the area.

Salinities in the anchorage ranged from 25.1 ppt. (Station 1, January, 1975) to 36.0 ppt. (Station 1, June, 1975). At Stations 1 and 2, salinities fluctuated more than at Stations 3 and 4, because of their proximity to the mainland and point sources of freshwater discharge. Salinities at Stations 1 and 2 increased steadily from December,

Table 13. Results of Kruskal-Wallis (H) and STP (U_s) non-parametric tests applied to mean test ratios averaged for 12 months. Tabulated H value is taken from Rohlf and Sokal (1969), Table R. N represents the number of sample means used for computing U_s. See Appendices 1 and 2 for complete computational methods.

KRUSKAL-WALLIS (H)

Station	Mean Annual Test Ratio	Range of Monthly Means	Standard Deviation
1	1.53	1.50-1.57	0.02
2	1.55	1.50-1.60	0.03
3	1.72	1.67-1.75	0.03
4	1.67	1.62-1.73	0.03

H = 11.89* Tabulated H = 7.82

STP

1-2	1-3	1-4	2-3	2-4	3-4	N
101	144*	144*	144*	144*	125*	12

Critical U_s = 116.5

*Significant at $P \leq 0.05$

1974 to high levels in June, 1975, and then decreased again to lows in October (Appendix Table 19). Freshwater runoff usually lagged behind periods of rainfall, and salinities at these stations did not reflect freshwater imput until a month after peak precipitation. At Stations 3 and 4, salinities were much more stable remaining between 30 and 34 ppt. through most of the year (Appendix Table 19). However, between August and October, freshwater runoff began to be reflected at these stations, and by fall, salinities had decreased to around 26 and 27 ppt.

The effect of thermal addition from power plant discharge is indicated in Figure 7. Water temperatures at Station 2 were generally 1-2^{o}C higher than those at Station 1. Differences between the stations were most noticeable between May and August, when normal seasonal temperatures were highest. During this period, a corresponding increase in urchin mortality was observed at Station 2. In August, as water temperatures often exceeded 32^{o}C, recently denuded tests were frequently encountered in the collections. At Station 1, urchins appeared to be in good health throughout the summer as temperatures generally remained around 30^{o}C.

Trends in mean oocyte diameter are compared with annual water temperature patterns in Figure 8. Mean oocyte diameters increased with rising temperatures early in the year and then decreased (spawning) about a month prior to the high summer temperature plateaus. They remained relatively small throughout most of the summer, and then in September,

just prior to decreasing fall temperatures, increased again. As temperatures fell rapidly during the fall, mean oocyte diameters declined to those typical of gametes in spent and recovering stages of development.

Figure 7. Bottom water temperatures for Stations 1 (solid line) and 2 (dotted line).

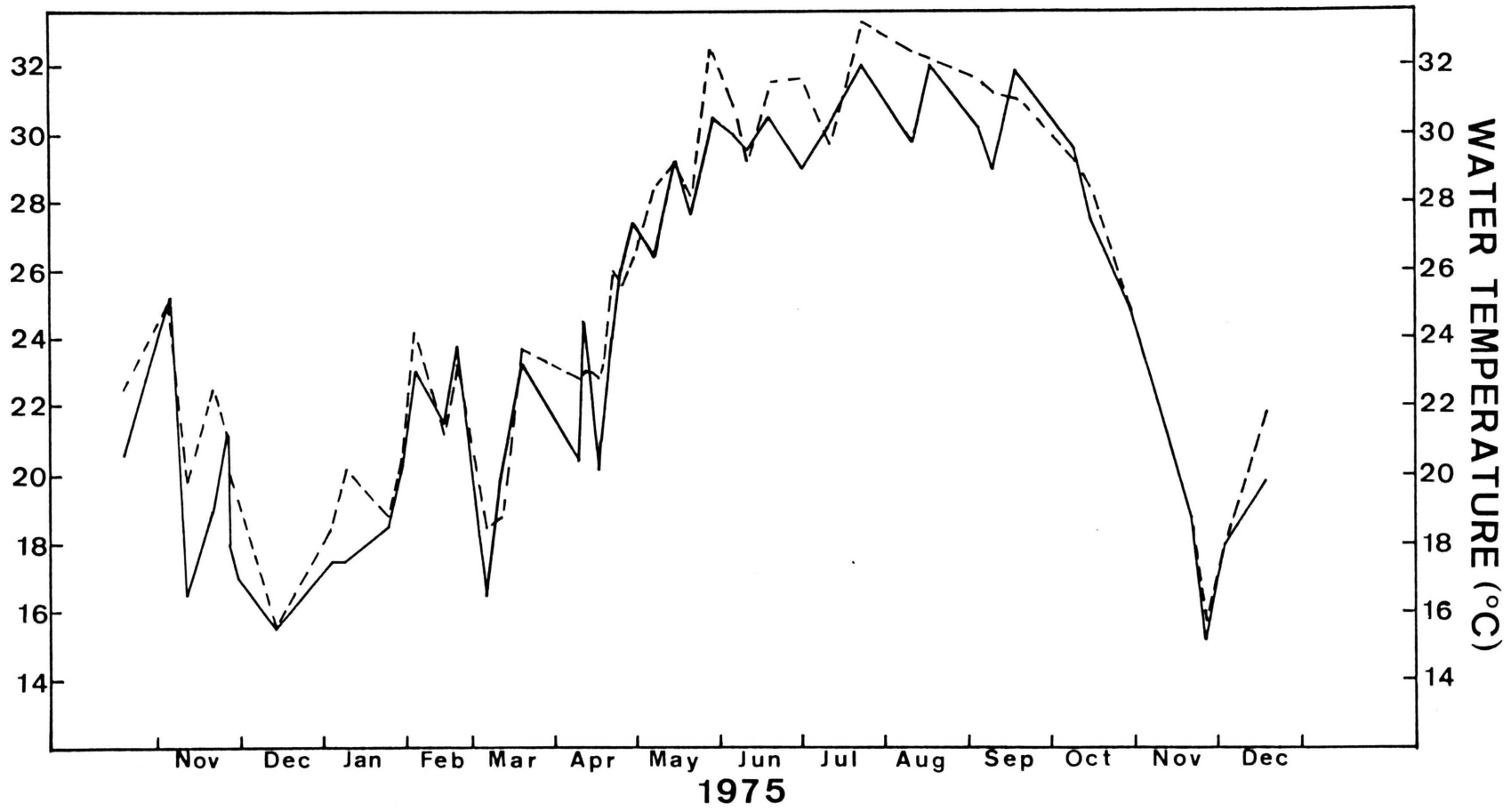
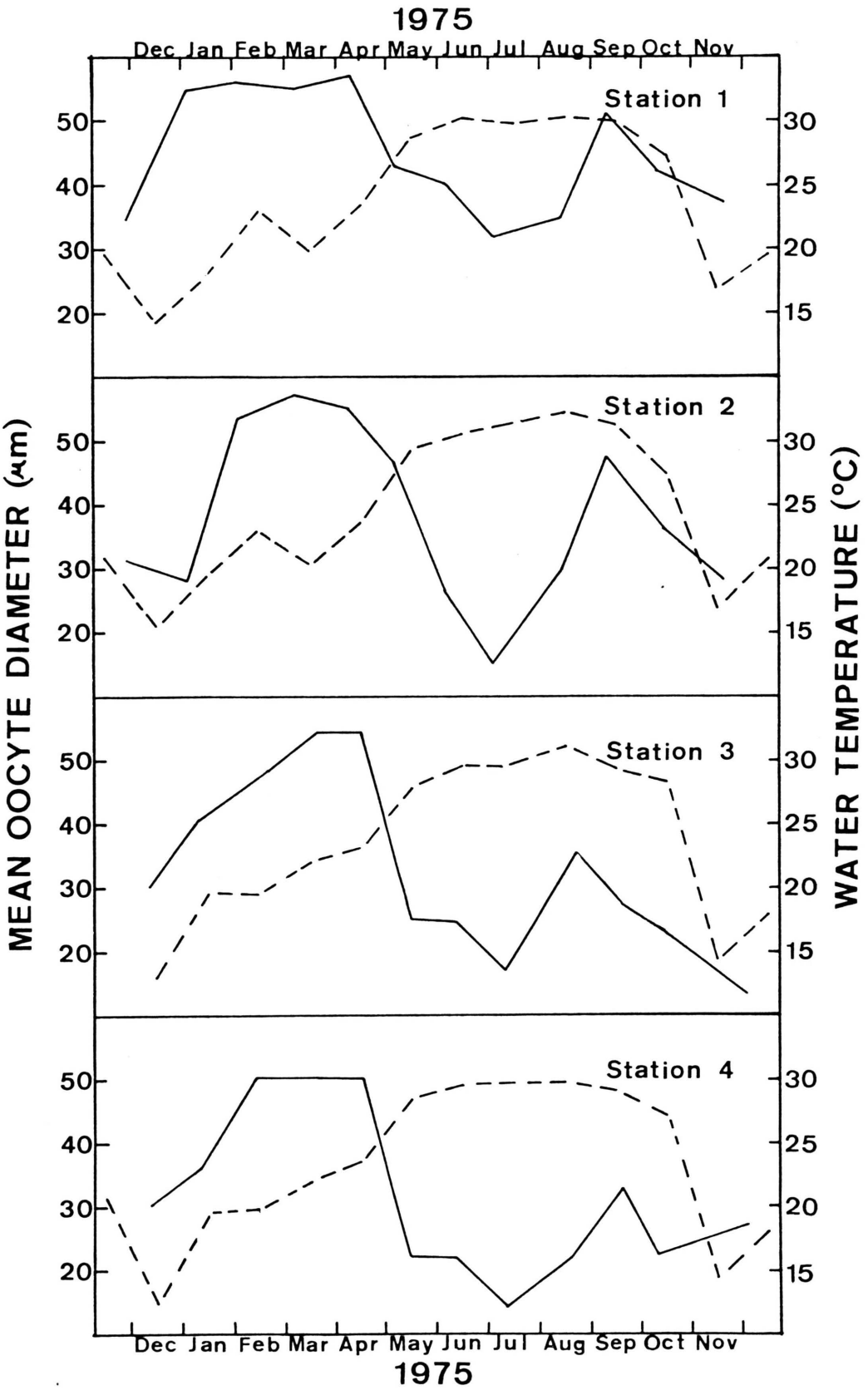

32
30
28
26
24
22
20
18
16
14
WATER TEMPERATURE (°C)
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1975

Figure 8. Relationship between ovarian gametogenic cycle (represented by mean oocyte diameter - solid line) and mean monthly bottom water temperature (dotted line).


1975
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
Station 1
Station 2
Station 3
Station 4
MEAN OOCYTE DIAMETER (μm)
WATER TEMPERATURE (°C)
50
40
30
20
30
25
20
15
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
1975

DISCUSSION

Information concerning the time of spawning has been reported for populations of Lytechinus variegatus ranging from the West Indies to the Carolinas (Table 14). Populations of Lytechinus at higher latitudes appear to commence spawning slightly later in the year than those at lower latitudes. Thus, in Miami and the West Indies, spawning reportedly commences in February and March, while along the Carolina coast, gametes are not shed until April and June, respectively. A similar spawning pattern exists along the Gulf coast of Florida, where urchins at Anclote appear to spawn a month or two earlier than those collected farther north. These findings are consistent with trends produced by organisams having thermally entrained reproductive processes (Orton, 1920; Giese and Pearse, 1974; Sastry, 1975). However, analyses of 10 years of reproductive data have failed to find a correlation of water temperature with time of spawning (Moore and Lopez, 1972).

Lengthened breeding at lower latitudes is another trend generally expected for organisms having thermally regulated reproductive cycles (Giese and Pearse, 1974). Moore et al. (1963) reported that spawning of Lytechinus at Miami was continuous with some reproductively ripe individuals being

Table 14. Latitudinal variation in the time of initial spawning for populations of Lytechinus variegatus

Locality	Time of Spawning	Source
Carolina Coast	June-July	Tennent (1910)*
Bermuda	April-May	Moore et al. (1963)
North Florida, Gulf Coast	April-May	Brookbank (1968)
Central Florida, Gulf Coast	March	Present Study
South Florida	February-Marh	Moore and Lopez (1972)
West Indies	March-April	Mortensen (1921)*

*Taken from Boolootian (1966), Figure 25-22, p. 581.

collected throughout most of the year. Spawning at Anclote began in early spring and continued at a reduced rate throughout the summer. At Bermuda, spawning was much more abbreviated, lasting only two months (Moore et al., 1963). Thus, southern populations of Lytechinus appear to breed over a longer period than more northern populations.

Organisms breeding over shorter periods usually have more intense spawning peaks than those with continuous breeding patterns (Sastry, 1975). A conversion factor was computed to allow for comparisons between volumetric and dry weight gonad index values. The data indicates that for 1975, peak gonad production at Anclote was considerably lower than average annual highs at either Miami or Bermuda. Since urchins at Anclote have a shorter spawning period than those at Miami, the reverse situation might be expected. Several explanations may account for the disparity.

Environmental variables are known to influence reproduction temporally, and it is possible that the 1975 reproductive cycle of Lytechinus at Anclote may have been unrepresentative of normal reproductive behavior. Moore and Lopez (1972) found that higher than average rainfall (and therefore lower salinities) was associated with smaller than average full gonads. At Anclote, monthly precipitation from October, 1974 to May, 1975, the period including gonad growth and initial spawning, was averaged to determine if rainfall exceeded normal amounts. Precipitation during that period was about one inch below normal. If the correlation found at Miami

applies equally to Anclote, the peak index for 1975 may actually have been slightly higher than average.

Another source of error may arise from the methods used to arrive at gonad index values. Giese and Pearse (1974) have suggested that dry weight data best serve gonad index studies because they alleviate discrepancies attributable to fluctuations in water content of tissues. Bernard (1977) indicated that in Stronglocentrotus franciscanus, there is a certain amount of water uptake immediately prior to spawning, resulting in a decrease of dry weight to wet weight ratios. A similar situation would exist for dry weight to volume ratios, so that at peak gonad maturity, dry weight to volume ratios would be lower than they are at other times during the reproductive cycle. Since the conversion factor was not calculated at the time of peak gonad development, it is uncertain how the ratios for Lytechinus may have been affected. It seems likely however, that volumetric gonad index values reported for Miami populations may have overestimated peak gonad development.

A final explanation might be in the use of urchins having different size frequency distributions. Urchins with test diameters of 52-55 mm. would be expected to yield higher index values than those larger than 55 mm. (Moore et al., 1963). All sizes of urchins were used in gonad index calculations at Miami, while only those individuals between 55 and 65 mm. were used at Anclote.

It becomes apparent that intraspecific reproductive variability among geographically separated populations is difficult to infer by comparing data generated from short term studies. This is due primarily to the large degree of annual variability possible among local populations. Moore and Lopez (1972) found spawning commencement dates to differ by nearly four months and the size of full gonads to vary two fold for populations of L. variegatus followed for several years at Miami. Other problems arise when spawning periods are deduced from gonad indices. Gonad indices may not necessarily reflect actual gametogenic processes, and habitat can play a decisive role in determining how well gametogenic processes and gonad growth curves are aligned.

The annual reproductive cycle depicted for sub-populations of Lytechinus at Anclote is typical of echinoids and has similarly been reported for populations of Lytechinus studied at other locations (Boolootian, 1966; Moore et al., 1963; Moore and Lopez, 1972). Within the framework of the annual cycle, habitat measureably influences the phenotypic expression of reproduction as monitored by the gonad index. However, when information generated by the gonad index is compared with histological data, a rather uniform trend of reproductive activity emerges.

During the present study, synchrony in timing of reproductive events can best be inferred from data derived from histological indices. Gonad maturity and mean oocyte diameter curves indicated two relatively synchronized peaks of

advanced activity. These peaks corresponded to periods when relatively high numbers of ripe individuals were found in the collections and thus substantiate a bimodal reproductive cycle. It is apparent that the major spawning occurred in spring and represented the principal reproductive effort for urchins at most stations. The late summer - early fall spawning period marked the end of the annual cycle. Subsequently, oocyte production ceased and phagocytic coelomocytes lysed all remaining tissue within the follicles. This final cleansing was universal among sub-populations and may serve as an agent to bring the population back into gametogenic synchrony. Gonor (1973) similarly found for S. purpuratus that the proliferation period of a new reproductive cycle did not begin until the delayed maturation and spawning of remaining large oocytes and ova of the previous cycle had been completed. It is likely that an external factor may prompt the summer maturation and spawning, but identity of the cue is obscure. Another synchronizing agent in the reproductive cycle of Lytechinus apparently initiates the spring spawning.

Spawning commencement may be inferred from decreases in any of the indices used to monitor gonad growth and gametogenesis. The curves generated from mean monthly gonad index, mean monthly gonad maturity and mean oocyte diameter data variously denoted the commencement of spawning. The only data which suggested that spawning may have begun simultaneously at all stations was obtained from mean monthly

oocyte diameters. However, mean oocyte diameters cannot be used to differentiate between advanced oocytes and mature ova, and thus this index is of limited value in detecting the precise commencement of spawning. Declines in mean monthly oocyte diameters do serve to mark the end of advanced gametogenic activities, signifying that after April, the number of young primary oocytes and oogonia relative to the number of advanced oocytes maturing into ova had increased substantially. The attenuation of major spawning activities in May was also indicated by both gonad index and gonad maturity data. Thus, eventhough spawning may have begun at slightly different times, a relatively synchronous period of gamete dispersal did exist. Within the period, spawning was probably episodic involving limited groups or sub-populations similar to the phenomena reported for other echinoids (Boolootian, 1966; Bernard, 1977). Spawning synchrony among local populations inhabiting heterogeneous environments has previously been reported for the echinoids Strongylocentrotus nudus and S. intermedius (Fuji, 1960b), Echinometra lucunter (McPherson, 1969), Evechinus chloroticus (Dix, 1970), S. purpuratus (Gonor, 1973), Dendraster excentricus (Niesen, 1977), and S. franciscanus (Bernard, 1977).

Following the major spring spawning, gonad index curves assumed a variety of shapes in response to continued spawning and presumably differential rates of gametogenic recovery. Histological indices did indicate a period of relative reproductive inactivity during the summer. However, low points

in gonad maturity and mean oocyte diameter curves cannot be described as quiescent periods. Even immediately following the initial spring spawning, unshed ova of Stage V urchins were quickly resorbed by nutritive phagocytes refilling the vacated lumen. During this period, new oocytes were continually being produced, and on no occasion did oocyte production cease ensuant to a complete lysing of tissue within the follicle such as occurred after the fall spawning. Thus, similar to findings in other areas of Florida, Lytechinus at Anclote may have the potential to spawn over a relatively long portion of the year (Moore et al., 1963; Brookbank, 1968).

Gametogenesis in L. variegatus proceeded in a manner closely agreeing with those described for other regular urchins (Fuji, 1960a); Holland and Giese, 1965; Chatlynne, 1969; Gonor, 1973; Bernard, 1977). Oocytes in various stages of development attained dimensions and assumed positions in the follicle typical of these echinoids.

The principal difference among sub-populations of Lytechinus at Anclote was related to the mean relative amounts of gonad material present each month. Consequently, annual gonad index curves differed markedly. Since gametogenic patterns were comparatively uniform among sub-populations, the alignment of gonad index curves with curves derived from histological indices also differed substantially.

It should be noted that replicate samples were not taken at any station during the course of study. Thus, if variability among individuals within a station was sufficiently

large, two samples from the same location may have yielded significantly different results. Boolootian (1966) has recommended using 15 specimens when studying reproduction in sea urchins. Although many researchers have used sample sizes of 50, studies using lesser numbers (20-30) have provided equally convincing results regarding reproductive variability among urchins from different habitats (Gonor, 1972 and 1973). Furthermore, sample sizes of 25 are more than adequate for statistical comparisons using non-parametric tests. Therefore, depicted patterns of gonad development at Anclote are thought to be characteristic of the particular habitat sampled.

In most echinoids where both gonad index and histological data have been reported, maximum gonad size corresponds to the period when the highest percentage of ripe individuals occur in collections (McPherson, 1965; Dix, 1970; Gonor, 1973; Niesen, 1977). During the present study, gametogenic cycles and gonad index curves were closely aligned during the initial gonad growth and spawning phases. However, depending on location, secondary gametogenic activities occurring later in the year were variously reflected by gonad growth curves. The incongruous nature of the alignment of these curves is probably related to differences in patterns of nutrient accumulation and clearly denotes the influence habitat can exert on phenotypic expressions of reproduction. As Gonor (1973) states, "the process of nutrient accumulation and gametogenesis are interrelated but their initiation and

rates may be influenced independently by environmental factors."

In urchins, the initial increase in the gonad index usually results from nutrient accumulation, and gamete production begins only after an adequate nutrient reserve has been established (Pearse, 1969; Gonor, 1973; Bernard, 1977). Such a situation was most obvious at Station 2, where the gonad index reached its highest peak two months prior to the spring peak in the gonad maturity index. A similar pattern also existed at Station 1 later in the year, where from May to July, the gonad index curve increased well in advance of corresponding rises in histological curves. At the other stations (including Station 1 in the spring), gonad maturity indexes seemed to parallel the increase in gonad weight indicating that nutrient accumulation and gamete production proceeded simultaneously or that the events were not separated sufficiently in time to be distinguishable with the monthly sampling schedule.

Since the spawning patterns of Lytechinus at Anclote are comparable among sub-populations, differences in spawn production should be related to differences in the quantities of nutritive material available for incorporation into reproductive products. Such a case existed for the urchin S. purpuratus, where the total number of eggs produced during its spawning period was related to the maximum prespawning size of gonads (Gonor, 1973). Accordingly, urchins at Station 2 would be expected to yield the greatest number of

gametes during the spring spawning. However, as previously noted, spawning may continue over a relatively long portion of the year, and if gonad quantity is a good estimator of spawn production, the areas under the gonad index curves may provide a better gauge for comparing differences among stations. Since, no significant differences were detected among stations, it must be assumed that the potential for gamete production was uniform among urchins from different habitats. High gonad index values for urchins at Stations 2 and 4 at the beginning of the reproductive cycle, apparently offset the absence of summer growth phases there. Of course, this assumes that all gonad material was converted into gametes during the course of the reproductive cycle. Due to the competitive interaction of phagocytic and gametogenic processes, some resorption is always taking place (Holland and Giese, 1965; Chatlynne, 1969). Furthermore, during times of low food availability or increased metabolism, normal physiological requirements may draw on stored nutrients from the gonads (Ebert, 1968). Thus, eventhough the relative quantity of gonad material did not differ significantly among stations, the actual annual spawn production may have. Considering the appreciable differences among areas under the curves, it is also possible that the non-parametric test used was simply not sensitive enough to detect differences.

At the end of the reproductive cycle, urchins at Stations 3 and 4 had significantly smaller spent gonads than did urchins at Stations 1 and 2. It is possible that higher

spent values are related to greater food supply. A larger amount of residual gonad material following spawning may provide a "head-start" on the next annual reproductive cycle, whereby less nutrient accumulation is required to attain maximum gonad size. This would allow for greater energy channelization to test growth during the quiescent portion of the gametogenic cycle and may provide a buffer against possible adverse environmental conditions during the ensuing cycle.

Stations 1 and 2 located in similar habitats provided an opportunity for assessing the effects of temperature on gonad growth. During the winter, the gonads of urchins at Station 2 reached maximum size two months prior to those of urchins at Station 1. It is possible, that during the winter, above-ambient temperatures at Station 2 may have increased feeding rates thus allowing for an earlier channelization of energy to the gonads (Moore and McPherson, 1965). Since gonads of urchins at Station 2 attained a relatively larger size, the potential for spawn production was also greater. During the summer, as ambient water temperatures approached annual maxima, thermal discharge from the power plant had an adverse effect.

During most of the year, the flora of Stations 1 and 2 appeared to be quite similar. However, above-ambient summer temperatures at Station 2 reduced both the quantities and types of algae found there. At the time of highest water temperatures (July and August) seagrass cover was also

noticeably reduced, turbidity increased and bluegreen algal mats began covering the area. Since a mixed diet of plant material appears to be of greater nutritional value to Lytechinus than a monospecific one (Lowe and Lawrence, 1976), temperature may have indirectly affected reproduction by reducing the available choices of food. Furthermore, those items which were available may not have been appropriate to the urchins mode of feeding (Lawrence, 1975a). Additionally, increased summer temperatures at Station 2 may have affected reproduction by increasing metabolism (Moore and McPherson, 1965), reducing activity coefficients (Lawrence, 1975b), and retarding feeding (Moore and McPherson, 1965). An energy deficiency probably resulted, and the energy which was obtained was channeled to processes other than reproduction. The overall effect was the noticeable lack of a summer gonad growth phase at Station 2.

The bimodal index curve characteristic of urchins at Station 1 is unusual among echinoids. Goand index curves of populations of Lytechinus at Miami have shown considerable annual variation, but distinctly bimodal patterns have not been reported (Moore and Lopez, 1972). When urchins have shown distinct winter and summer spawnings (McPherson, 1965; Sastry, 1975), these patterns have been demonstrated to be phenotypic in nature (Sastry, 1966 and 1975). It is suggested that the trends at Anclote are also phenotypic expressions of the particular environments acting on reproductive activities. However, since histological indices

suggest that a bimodal spawning pattern is universal for urchins at Anclote, it seems likely that a bimodal gonad growth curve is a manifestation of optimum environmental conditions. Although it is difficult to infer what advantage is derived from such a pattern, it is clear that the summer buildup at Station 1 allowed for a potentially greater second spawning effort relative to other stations. Larval development often requires strict thermal regimes (Kinne, 1970; Andronikov, 1975), and a bimodal pattern may maximize spawn production during the two periods of the year when these conditions are present.

In summary, it may be said that assimilation and the subsequent channelization of energy to the gonads is dependent on the amount and nutritional quality of available food, the ease with which it can be acquired and ingested and the physiological demands of the urchins consuming it (Lawrence, 1975a). Each of the stations sampled during the present study had unique combinations of food and physical regimes which became manifest in different gonad growth patterns. These patterns may have affected the quantities of gametes produced over the year. Thus, reproductive success and ultimately population dynamics may be influenced by habitat. The need to collect succesive samples at the same location when studying reproduction in sea urchins or invertebrates in general is becoming increasing apparent.

In addition to the effect habitat may have on reproduction, differences in habitat have also been found to affect

the morphological disposition of urchin populations (Moore, 1934; McPherson, 1965). During the present study the shapes of urchin tests were found to be significantly different among sub-populations. Urchins in heavily vegetated areas (Stations 1 and 2) had the highest tests while those on barren substrates (Station 3) had the flattest. Generally, flat test shapes have been considered an ecological advantage in wave stressed areas where surge tends to upset the urchins. Thus, at Anclote, it appears that as vegetational cover increases, greater protection is afforded against currents or waves. Although Moore et al., (1963) found no change in test shape with age, it seems likely that test shapes are a phenotypic expression of the environment and may be expected to change with age for those individuals experiencing a change of habitats.

CONCLUSIONS

1. Populations of Lytechinus variegatus in the Anclote Estuary, Florida displayed a distinct annual reproductive ryhthum with gonad growth occurring in winter, initial spawning beginning in late winter-early spring and final spawnout and recovery taking place in fall.
2. Histological data suggest that a bimodal gametogenic pattern may be characteristic of urchins at Anclote, and a bimodal gonad index curve probably represents a manifestation of optimum environmental conditions.
3. Habitat strongly affects the reproductive activities of Lytechinus primarily through regulation of nutrient accumulation. Consequently, gonad growth curves may not necessarily reflect actual gametogenic patterns.
4. Two periods of snychronization apparently exist in the reproductive cycle of Lytechinus at Anclote. The agents responsible for synchronization are unknown but appear to induce spawning in mature individuals at the beginning of the cycle and prompt the maturation and spawning of advanced oocytes and ova at the end of the cycle.
5. Comparative reproductive data suggest that Lytechinus begins breeding slightly later in the year along a northerly latitudinal gradient with the length of

spawning period becoming correspondingly shorter.

6. Urchins from different habitats had significantly different test shapes. These differences appear to be related to the amount of vegetational cover present.

SUMMARY

1. Monthly collections from each of four habitats in the Anclote estuary, Florida provided reproductive data on Lytechinus variegatus in relation to environmental heterogeneity.
2. Gametogenesis in Lytechinus at Anclote appeared characteristic of processes previously described for other sea urchins.
3. Histological data suggest that two periods of advanced gametogenic activity are characteristic for urchins at Anclote. This bimodal pattern may not necessarily be reflected by gonad growth curves.
4. Differences in reproductive events among sub-populations are related primarily to patterns of nutrient accumulation which may be noticeably affected by existing environmental conditions. These differences are phenotypic in nature and relate to habitat variability.
5. The inadequacies of using only gonad indices or histological data when studying reproduction in urchins are apparent.
6. Reproductive synchrony present during the initial spring spawning was later eroded as continued spawning and recovery took place at differential rates. However, during

the final spawnout in fall, synchronizing agents prompted the maturation and spawning of advanced oocytes and remaining ova thus once again bringing the population into gametogenic synchrony.

7. Lytechinus at Anclote appears to have the potential to spawn over a relatively long portion of the year.
8. The annual reproductive cycle depicted for Lytechinus at Anclote was compared with data reported for geographcially distant populations of the species. Urchins from more northern areas appear to spawn later in the year and for shorter periods than do more southerly populations.
9. Differences in spawn output reported for Miami, Bermuda, and Anclote populations of Lytechinus may be attributable to the methods used to estimate relative gonad size. (i.e. gonad index).
10. Sex ratios at Anclote did not appear to depart from uniformity among any of the sub-populations studied. However, urchins from different habitats had significantly different shapes with test profiles increasing in relation to the amount of vegetational cover present.

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APPENDICES

APPENDIX 1

Explanation of Kruskal-Wallis (H) non-parametric statistic.

The Kruskal-Wallis (H) one-way analysis of variance is used for determining whether k independent samples are from different populations. It is the most efficient of the tests for k independent samples, having a power-efficiency of 95.5% when compared with the parametric F test (Siegel, 1956). The Kruskal-Wallis test avoids making the assumption concerning normality and homogeneity of variance implicit in the F test, and it does not require equal sample size.

The computation of H is accomplished by ranking each of N observations from all samples combined. The smallest value is given the rank of 1 and the largest the rank of N. Tied values are given the mean of the ranks for which they are tied. The sum of the ranks for all observations in each sample is then calculated and used in the following equation:

$$H = \frac{12}{N(N+1)} \left[\sum_{j=1}^{k} \frac{Rj}{n_j}^2 \right] - 3(N+1)$$

where N = the number of observations in all samples combined (Σn_j)

k = number of samples

n_j = number of observations in jth sample

R_j = sum of ranks in jth sample

$\sum_{j=1}^{k}$ = directs one to sum over the k samples

H is distributed approximately as chi square, with df = k-1 for sample sizes greater than 5 (Table R - Rohlf and Sokal, 1969). If the calculated H value is equal to or greater than the appropriate tabulated value, the null hypothesis is rejected, and the samples are considered to be from different populations. The Kruskal-Wallis test does not indicate which samples are different and another test needs to be used when significant differences are detected (See Appendix 2). For further details refer to Sokal and Rohlf (1969), page 388, or Siegal (1956), page 184.

APPENDIX 2

Explanation of the STP (U_s) non-parametric statistic.

The STP (U_s) non-parametric statistic is based on the Wilcoxon-Mann-Whitney (U) statistic which tests for significant differences between two samples. Unlike U, U_s permits comparisons between k independent samples. Because it requires equal sample size, adjustments had to be made to data collected during certain months of this study. When necessary, adjustments were accomplished by eliminating observations for samples in excess of n for the smallest sample size (See text for rationale).

The computation of U_s is accomplished by first ordering the observations for each sample from high to low value. Samples are then compared, a pair at a time, until all possible combinations of two samples (six in the present study) have been examined. This is accomplished by counting the number of observations in sample a which are lower in value than the highest value in sample b. Next, the number of observations in sample a which are lower than the second highest value in sample b are counted. This process continues until all n values in both samples have been compared. The score for all observations is then summed, tied values receiving a count of

½. The larger of the two quantities, N (sum or scores for n observations) or n^2 - N, is entered as U_s. Finally, U_s values for each station pair are compared with the critical value of U_s which is calculated as follows:

$$U_{\alpha[a,n]} = \frac{n^2}{2} + Q_{\alpha[a,\infty]} n\sqrt{\frac{(2n+1)}{24}}$$

where a = number of samples compared

n = number of observations within each sample

$Q_{\alpha[a,\infty]}$ = significance level for the studentized range for a groups and ∞ degrees of freedom (Table U, Rohlf and Sokal, 1969)

U_s values greater than the critical value are significant, indicating that members of the pair are from different populations.

The STP test is used only when it has been previously determined that at least one pair of samples are significantly different. The Kruskal-Wallis (H) test was used for determining if significant differences existed in the larger set of samples (See Appendix 1). For further details refer to Sokal and Rohlf (1969), page 396.

Appendix Table 15. Gonad index values and test ratios for urchins collected at Station 1. N represents the number of individuals used for computing these values.

COLLECTION DATE	GONAD INDEX ($\frac{\text{dry wt gonads}}{\text{total dry wt.}} \times 100$)				TEST RATIO ($\frac{\text{test diameter}}{\text{test height}}$)			
	N	Mean	Standard Deviation	Range	N	Mean	Standard Deviation	Range
11/29/74	25	4.26	2.44	0.52 - 8.93	24	1.51	0.07	1.39 - 1.71
1/3/75	25	4.81	2.17	0.47 - 9.13	25	1.57	0.08	1.43 - 1.71
2/3/75	25	5.66	1.89	2.58 - 9.57	25	1.53	0.06	1.43 - 1.67
3/6/75	25	6.68	1.93	2.62 -10.39	25	1.52	0.07	1.34 - 1.62
4/8/75	25	5.19	2.37	2.00 -12.59	25	1.54	0.09	1.39 - 1.65
5/6/75	25	2.65	1.23	0.73 - 5.31	25	1.53	0.07	1.39 - 1.68
6/5/75	25	4.54	1.37	1.66 - 7.50	25	1.51	0.07	1.33 - 1.67
7/2/75	25	5.29	1.75	2.13 - 8.24	24	1.55	0.07	1.37 - 1.68
8/12/75	25	4.93	1.24	2.93 - 6.68	25	1.51	0.07	1.38 - 1.66
9/9/75	25	4.05	1.47	1.46 - 8.16	25	1.56	0.09	1.41 - 1.69
10/9/75	25	2.51	2.04	0.53 - 8.30	25	1.50	0.11	1.33 - 1.83
11/9/75	25	3.09	1.89	1.09 - 7.77	25	1.55	0.08	1.41 - 1.77

Appendix Table 16. Gonad index values and test ratios for urchins collected at Station 2. N represents the number of individuals used in computing these values.

COLLECTION DATE	GONAD INDEX				TEST RATIO			
	N	Mean	Standard Deviation	Range	N	Mean	Standard Deviation	Range
11/29/74	25	5.92	1.50	2.74 - 10.07	25	1.53	0.09	1.40 - 1.74
1/3/75	25	8.79	1.81	5.57 - 13.15	25	1.57	0.06	1.46 - 1.69
2/3/75	25	8.26	2.25	4.87 - 11.82	25	1.58	0.07	1.46 - 1.70
3/6/75	25	7.85	2.61	3.81 - 13.28	25	1.50	0.07	1.39 - 1.66
4/8/75	25	4.68	1.56	1.87 - 8.03	25	1.51	0.09	1.36 - 1.69
5/6/75	25	3.29	2.51	0.83 - 11.19	25	1.58	0.08	1.44 - 1.74
6/5/75	25	2.87	1.98	0.27 - 7.71	25	1.54	0.06	1.44 - 1.65
7/2/75	25	2.04	1.74	0.31 - 6.70	25	1.55	0.06	1.46 - 1.65
8/12/75	23	2.04	2.14	0.31 - 9.67	23	1.56	0.09	1.33 - 1.70
9/9/75	23	2.26	1.32	0.18 - 4.94	23	1.60	0.09	1.43 - 1.77
10/14/75	21	1.25	0.68	0.52 - 2.98	21	1.55	0.07	1.46 - 1.73
11/19/75	25	3.12	1.50	1.02 - 7.01	25	1.55	0.06	1.41 - 1.65

Appendix Table 17. Gonad index values and test ratios for urchins collected at Station 3. N represents the number of individuals used for computing these values.

COLLECTION DATES	GONAD INDEX				TEST RATIOS			
	N	Mean	Standard Deviation	Range	N	Mean	Standard Deviation	Range
12/11/74	25	1.49	1.43	0.19 - 4.94	25	1.74	0.09	1.55 - 1.89
1/9/75	25	3.15	1.25	0.67 - 6.97	25	1.74	0.11	1.52 - 1.97
2/13/75	25	5.72	1.42	2.88 - 8.08	24	1.67	0.12	1.36 - 1.85
3/18/75	25	7.15	2.04	3.75 -11.62	24	1.72	0.09	1.53 - 1.84
4/16/75	25	5.67	1.70	3.02 - 9.92	25	1.73	0.08	1.56 - 1.87
5/14/75	25	4.47	2.29	0.61 - 8.99	25	1.75	0.10	1.58 - 2.00
6/10/75	25	5.14	2.26	1.14 -11.65	25	1.70	0,11	1.49 - 1.93
7/10/75	25	3.36	2.19	0.22 - 9.04	25	1.73	0.10	1.50 - 2.00
8/21/75	25	4.15	1.91	1.24 - 9.27	25	1.71	0.08	1.53 - 1.84
9/17/75	21	1.04	0.74	0.15 - 3.08	21	1.71	0.11	1.55 - 2.00
10/14/75	24	0.76	0.89	0.12 - 2.81	24	1.67	0.08	1.49 - 1.85
12/3/75	25	1.27	0.90	0.21 - 3.94	25	1.71	0.07	1.56 - 1.78

Appendix Table 18. Gonad index values and test ratios for urchins collected at Station 4. N represents the number of individuals used for computing these values.

COLLECTION DATE	GONAD INDEX				TEST RATIOS			
	N	Mean	Standard Deviation	Range	N	Mean	Standard Deviation	Range
12/11/74	25	3.15	1.41	1.17 - 7.01	24	1.73	0.11	1.55 - 2.03
1/9/75	25	3.73	1.62	1.54 - 7.40	25	1.69	0.09	1.55 - 1.91
2/13/75	25	7.83	1.82	3.83 -10.72	25	1.70	0.09	1.58 - 1.94
3/18/75	25	4.36	1.42	1.80 - 7.27	24	1.64	0.08	1.50 - 1.88
4/16/75	25	4.92	1.82	2.23 - 8.98	25	1.69	0.08	1.53 - 1.90
5/14/75	25	3.22	1.64	0.92 - 9.79	25	1.66	0.06	1.57 - 1.77
6/10/75	25	2.80	1.35	0.79 - 5.72	25	1.65	0.12	1.45 - 1.85
7/10/75	25	2.77	1.68	0.56 - 6.86	25	1.64	0.07	1.47 - 1.79
8/18/75	25	1.77	0.99	0.24 - 4.82	25	1.62	0.06	1.51 - 1.79
9/17/75	25	0.98	0.66	0.16 - 2.84	25	1.69	0.08	1.55 - 1.85
10/9/75	25	0.74	0.73	0.22 - 2.96	25	1.68	0.09	1.50 - 1.82
12/3/75	25	2.95	1.67	0.40 - 6.60	25	1.64	0.07	1.50 - 1.78

Appendix Table 19. Mean monthly temperature (°C) and salinity (°/oo) at four sampling locations in the Anclote Anchorage.

Month	Station 1		2		3		4	
	T	S	T	S	T	S	T	S
November 1974	19.8	27.2	20.9	27.5	-	-	20.7	-
December 1974	14.3	26.7	15.5	-	13.0	32.6	12.5	30.5
January 1975	17.9	26.8	19.4	28.8	19.5	32.4	19.3	32.4
February 1975	22.9	29.4	22.9	30.9	19.5	30.2	19.8	30.2
March 1975	19.8	30.2	20.3	-	22.0	33.1	22.0	33.3
April 1975	23.3	32.8	23.8	33.5	23.1	32.8	23.5	33.1
May 1975	28.6	34.4	29.3	34.5	27.8	33.7	28.4	34.5
June 1975	30.1	34.5	30.5	35.4	29.5	34.1	29.5	-
July 1975	29.8	33.2	31.4	33.7	29.4	33.2	29.7	33.0
August 1975	30.2	29.7	32.3	31.6	31.1	33.1	29.8	32.8
September 1975	29.9	29.4	31.2	31.1	29.3	30.4	29.2	30.1
October 1975	27.2	26.6	27.5	26.4	28.3	26.5	27.2	25.8
November 1975	17.0	26.8	16.9	27.7	14.5	-	14.5	-
December 1975	19.8	-	20.8	26.4	18.0	27.1	18.0	27.5