AN ABSTRACT OF THE THESIS OF Richard Donald Braley for the Master of Science in Biology presented April 21, 1981.

Title: Reproductive periodicity in the indigenous oyster Saccostrea cucullata in Sasa Bay, Apra Harbor, Guam, with reference to cultivation.

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The Saccostrea cucullata population in Sasa Bay underwent low-level continuous reproduction with three main peaks per annum. Spawning activity occurred in November-December, March-April, and late June. Gametogenic cycles took 3-4 months to complete. Histological examinations revealed the general trend of gametogenesis to be similar for both sexes, although they were not always in synchrony. Partial spawning with resorption of unspawned gametes was the general rule. Neither temperature, salinity, turbidity, nor climatological parameters appeared to be exogenous clues for spawning. Evidence of lunar periodicity in spawning was not apparent from histological examinations.

Peaks in spat collection generally followed peaks in gonad ripeness by one month or less which indicates that the planktonic larval period lasts about 3-4 weeks. There were significant differences in the abundance of spatfall between sites but not between collector types. Metamorphosing larvae preferred concave surfaces over convex.

Oyster larvae were a major component of bivalve larvae taken in zooplankton tows. Main peaks of larval abundance were in May, October, and November-December. The relative abundance of oyster larvae in the vicinity of an oil spill was reduced for several months. Histological examinations of adult oysters taken near the oil spill site revealed an occurrence of 0.77% sex-reversal/hermaphrodism pre-spill and 2.78% post-spill.

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REPRODUCTIVE PERIODICITY IN THE INDIGENOUS OYSTER *SACCOSTREA CUCULLATA* IN SASA BAY, APRA HARBOR, GUAM WITH REFERENCE TO CULTIVATION

by

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INTRODUCTION

A knowledge of the timing and intensity of reproduction in marine organisms is central to an understanding of their life history, ecology, and suitability for cultivation. Although little quantitative information on reproductive behavior is available for tropical species, the exogenous factors which initiate and synchronize the timing of their reproduction appear to differ from those at higher latitudes (Johannes 1978). Major factors include variations in temperature, photoperiod, plankton density, and salinity (Giese and Pearse 1974). Lunar rhythms superimposed on such parameters have been implicated as exogenous factors which affect reproduction in some marine organism (Orton 1926; Korringa 1947, 1957; Johannes 1978).

Studies on the reproductive cycles and spawning of oysters and mussels have been documented by several researchers (e.g., Coe 1931, 1932; Loosanoff 1942; Rao 1956; Durve 1965; Seed 1969; Ling 1970; Stephen 1980), often with special reference to cultivation of the species. Many arguments have been advanced for the use of local rather than exotic species for mariculture (Braley 1978; Bourne 1979; Newkirk 1979; Quayle 1980). Therefore, a study on the reproductive cycle of the indigenous Guam mangrove oyster, Saccostrea cucullata (Born) was initiated. This species is the only intertidal oyster on Guam and it occurs within the shallow areas of Apra Harbor (i.e. Sasa Bay, Piti Channel, Inner Harbor) at a population density of approximately 2500/m^2 (Amesbury et al. 1977).

It grows in profusion on the prop roots of mangroves, Rhizophora spp., and on intertidal rocks and wrecks.

Saccostrea cucullata is a widespread Indo-Pacific species ranging from East Africa to the Pacific islands. Spawning in this species was found to be continuous except for the monsoon time in India (Awati and Rai 1931), continuous but peaked during the rainy season in East Africa (Van Someren and Whitehead 1961), and continuous with three peaks in Singapore which generally followed the monsoon periods (Ling 1970). Although this literature provides leads to understanding the life history of this species on Guam, the reproductive periodicity may vary with locality (Quayle 1980). Therefore, the object of the present work was to quantify reproduction in populations of Saccostrea cucullata on Guam over an annual cycle. The potential of the local oyster for mariculture depends in part on the magnitude and seasonal occurrence of spawning and spatfall. To this end, the spatfall and larval density in the plankton were examined in conjunction with a histological study of gametogenic cycles and selected environmental parameters.

MATERIALS AND METHODS

Histological Preparations

Approximately 30 adult specimens of Saccostrea cucullata were collected monthly from September 1979 through October 1980 from a population on intertidal rocks in the vicinity of the Laguas River (Figure 1). All collections were made on or near the day of the full moon. The oyster tissue was preserved in Bouin's fixative. A biopsy was done on preserved specimens by removing a small piece of gonadal material from the extreme posterior end of the gonad to determine sex and relative gonad condition. A combined total of 24 oysters per collection was randomly selected for histological examination. The October 5, 1979 and February 1, 1980 collections had 20 and 23 specimens, respectively. The gonad was transversely sectioned, and the anterior half was embedded in paraffin and sectioned at 10 µm, in accordance with routine procedures (see Humason 1967). Two slides with four serial sections separated by about 0.3 mm of tissue were prepared for each individual. These were stained with Delafield's hematoxylin and eosin.

Eight stages of gametogenesis were defined by examination of the prepared slides. Gonad condition was classified according to a modified version of Seed's (1969) scheme. The stages recognized were:

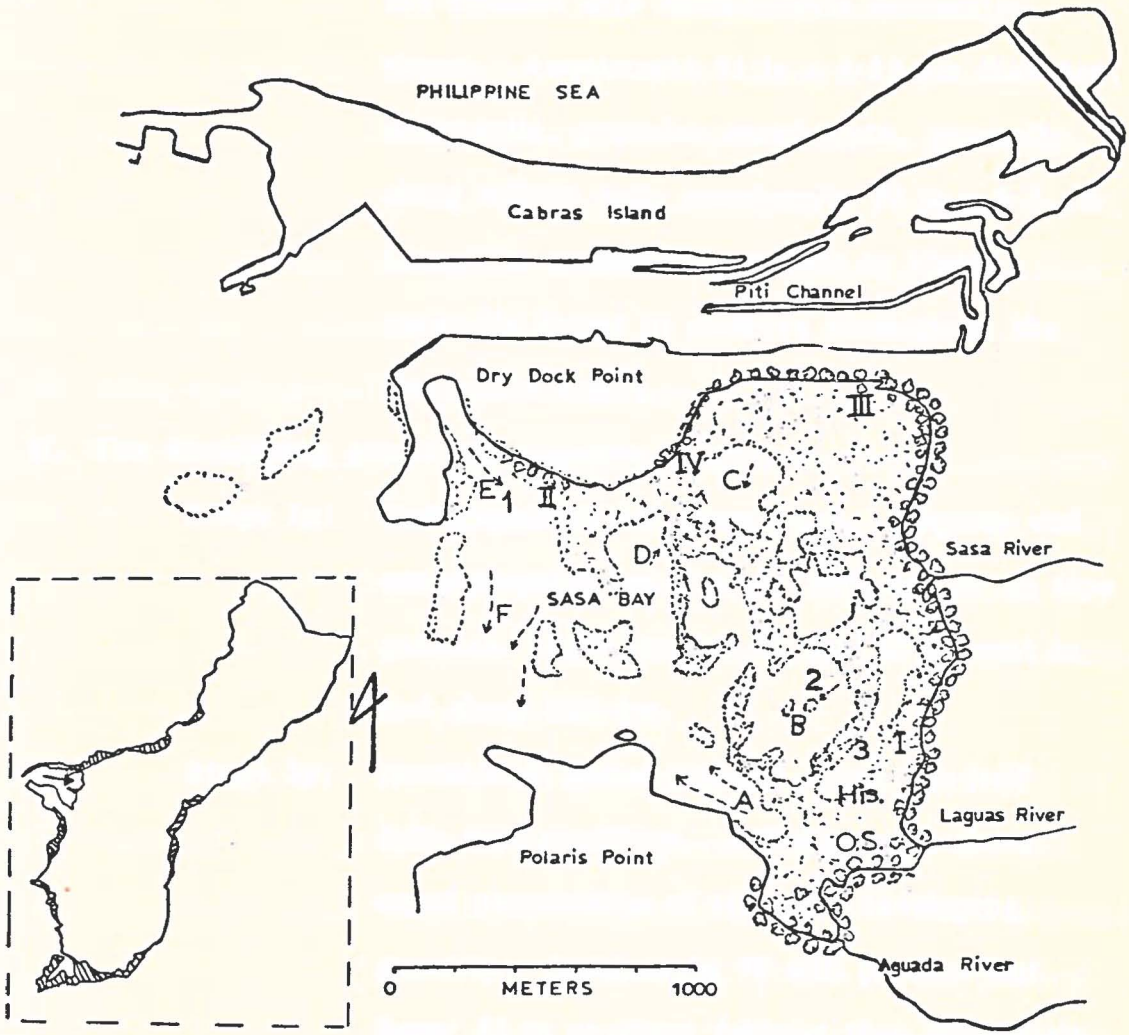

Figure 1. Map of study area. Patch reefs are enclosed by dotted lines and mangroves are drawn along shore. Inset map of Guam included. His. - oyster bed for histology samples; I-IV - spat collection sites; A-F - current study stations with arrows indicating approximate direction of water movement; O.S. - oil spill site. (modified from Amesbury et al. 1977)

I. The resting gonad

Stage O: Inactive, neuter. Amoebocytes #1 and #2 are present with considerable connective tissue. Amoebocyte #1 is a 6-13 μm diameter, acidophilic, granular amoebocyte, generally along follicle wall; amoebocyte #2 is a 2-3 μm basophilic amoebocyte, brown or black, generally found in clusters throughout the connective tissue.

II. The developing gonad (progressive stages)

Stage 1p: Gametogenesis has commenced. Oogonia and spermatogonia are present with few or no ripe gametes. Amoebocytes #1 and #2 present in and along follicles.

Stage 2p: Increase in gonad mass to about one-half fully ripe condition. Follicles have about equal proportions of ripe and developing gametes. Amoebocyte #1 still present but fewer #2 in or along follicles than previously.

Stage 3p: Gonad as great as two-thirds or more of final size. Mostly ripe gametes in follicles but gametogenesis is still progressing. Reduced connective tissue and amoebocyte #1 may still be present along follicles.

III. The ripe gonad

Stage 4: Gonad fully ripe. Early stages of gametogenesis reduced and ova packed into polygonal formation, or follicles distended with ripe sperm. Cytoplasm of mature ova filled with yolk (vitellogenesis complete). Amoebocyte #1 may still be observed occasionally along outer follicle wall.

IV. The spent gonad (regressive stages)

Stage 3r: Post-spawning. Partially emptied gonads with amoebocytes #1 and #2 generally present along follicles. Interfollicular connective tissue may begin to reappear. Gametogenesis is generally very reduced.

Stage 2r: More advanced post-spawning/regressive stage. Residual gametes remain in the follicles. Interfollicular connective tissue present again and amoebocytes #1 and #2 are present in or along follicles. Gametogenesis generally stopped or greatly reduced.

Stage 1r: Very advanced regressive stage. Very few residual gametes remain in follicles and there is a great increase in connective tissue. Amoebocytes #1 and #2 are present in and along follicles. Gametogenesis not evident.

The mean gonad index of Seed (1969) was calculated for each sample. This was determined by multiplying the number of oysters in each stage by the numerical rank of the stage; the sum of these products was then divided by the total number of individuals in the sample. The index may vary from zero, when the entire sample of the population is resting, to four when all are fully ripe. An increase in the index shows development and a subsequent decrease indicates either spawning or resorption. In addition, ratios of the gonad section width to diameter were obtained for two planes in each oyster section. These ratios were then compared with the stages determined by histological examination.

Throughout the study 179 males and 318 females were collected (1 male: 1.78 females). Of these, 156 males and 194 females were used in histological preparations. There was a mean height (3.72 ± 0.2 cm) and mean total wet weight (13.6 ± 1.6 g) of oysters used in the histological studies. The mean percent wet meat weight out of total wet weight was 17.9 ± 1.6%.

Oil Spill

A Guam Oil and Refining Company (GORCO) oil spill (Diesel Fuel Marine, 10,000 gal. estimated by the Coast Guard) was first discovered in early June 1980. This spill resulted from a leak in a pipeline near the mouth of the Laguas River (see Figure 1). The oil spill was located about 250 m from the oyster bed from which specimens were collected for the histological study.

Spat Collection

Spat collectors were set up at four locations in Sasa Bay (Figure 1). All of the stations were among mangroves near the shore. The substrate in the vicinity of station 1 was composed of more mud than the sandy-silt substrate found at the other three stations. Collectors were replaced on the tenth day of each month and examined quantitatively for spat. Spat collection began in November 1979 and continued through January 1981. Spatfall was quantified within a 30 cm height in the intertidal zone of Sasa Bay, since most of the successful spatfall occurs in this area. Two types of collectors were used; split bamboo stakes and plastic corrugated roofing material. The collectors were oriented perpendicular to the substrate. The bamboo stakes were set directly into the substrate while the plastic and plexiglass collectors were tied to rebar anchored in the substrate. Plastic roofing had six large corrugations and fourteen small corrugations for comparison. Flat plexiglass squares were set out monthly at stations I and IV.

Zooplankton Samples

Collection of zooplankton samples in Sasa Bay was made at three sites (see Figure 1). A plankton net with a diameter of 24 cm and a mesh of 100 μm was used for replicate 2.5-minute tows. The speed of the tow was determined by timing replicate fluorescein dye patches over the length of the boat. The plankton samples were immediately preserved in 4% formalin. From mid-March 1980 through February 1981 daytime tows were taken at each full and new moon. A single tow was taken in February 1980. A plankton-sample splitter

was used to obtain and count replicates of 1/32 of the volume of each tow sample. Bivalve larvae were grouped into nine categories according to size and shape of the larval shells.

Physical Parameters

Surface water temperatures and water samples were obtained at the times of plankton sampling and when spat collectors were changed. Collections were generally made between 1400-1700 hours. Turbidity, read in Nephelometric Turbidity Units (NTU), was determined with a Hach Turbidometer, and salinity was determined with a refractometer, generally less than six hours after sample collection. Climatological data on precipitation and percent of possible sunshine (determined as portion of the daylight hours when the sun is not obscured by clouds) were obtained from NOAA information service data for Guam (NOAA 1979, 1980).

Current Study

A current study was carried out in Sasa Bay on 29 July 1980, when the low tide was at a maximum for 1980 (-0.5 ft or -0.2 m; tide low at 1444). An estimate of the rate of movememt of water flowing out of the bay from selected areas was made to support the assumption that the oyster larvae in the zooplankton samples were derived from spawnings of oyster populations in Sasa Bay, and not elsewhere. One meter cloth drogues were used, and rates of movement recorded from four hours before low tide to one hour after low tide. An anemometer was used to record wind speed. Six stations were sampled.

RESULTS

Reproductive Periodicity

A plot of the mean gonad indices (M.G.I.) over a period of fourteen months (fifteen full moons) for males, females, and those oysters of indistinguishable sex is shown in Figure 2. This composite diagram also displays percentage of developing (stages 1p-4), spent (stages 3r-1r), and resting (stage 0) categories for each sample. Three prominant peaks in the M.G.I. occur in November-December, March-April, and late June, and these indicated spawning activity. The percentage of developing and spent individuals is consistent with the pattern seen in the M.G.I. plot. This indicates that although there are several peaks in activity, gametogenesis and spawning occur throughout the year.

The changes in gonad condition of female, male, and combined sex categories are shown in Figure 3. The stages of gonad ripeness are presented and serve to further support the contention that continuous reproduction occurs at a low level throughout the annual cycle. Figure 3 depicts the progression and regression of gonad condition through the months and indicates that smaller peaks of spawning occurred in February and August. The period of time between major peaks was 3-4 months. Males and females were not always in synchronous stages of ripeness. However, the general trends of gonad development and regression were similar for both sexes. The mean gonad ratio is significantly correlated ($r=0.9541$)

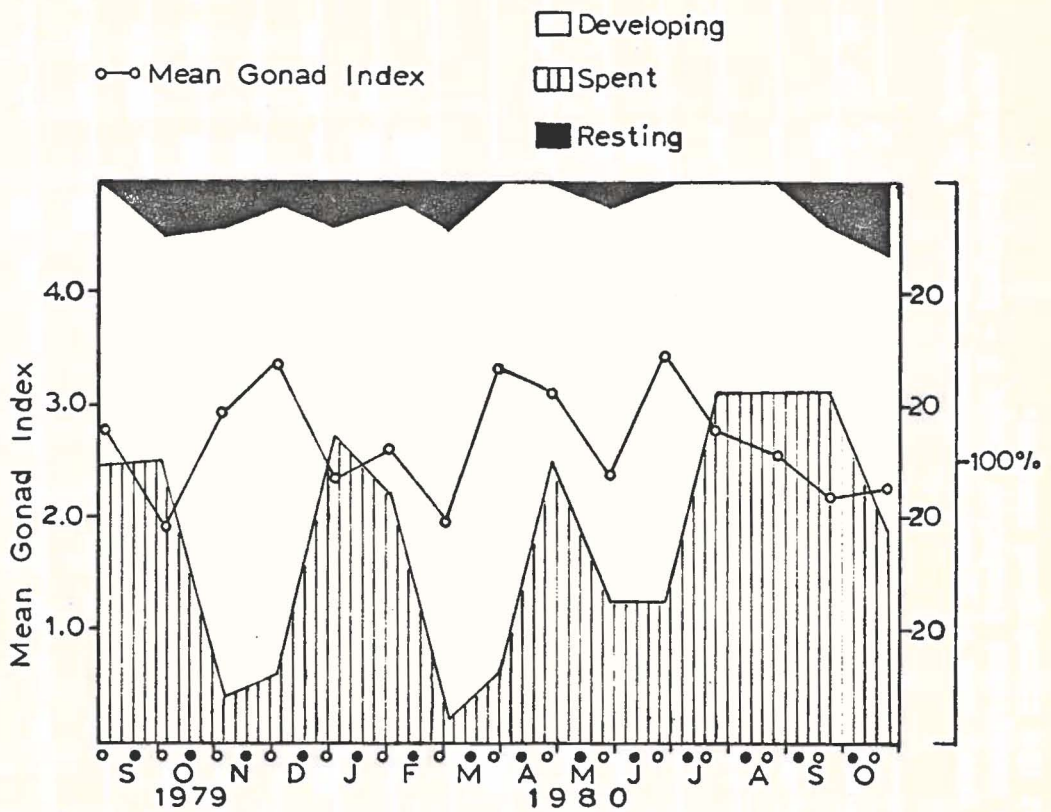

Figure 2. Composite diagram of the mean gonad index and percentages of individuals in three categories of the gametogenic cycle of Saccostrea cucullata. Full and new moon phases are indicated in each month.

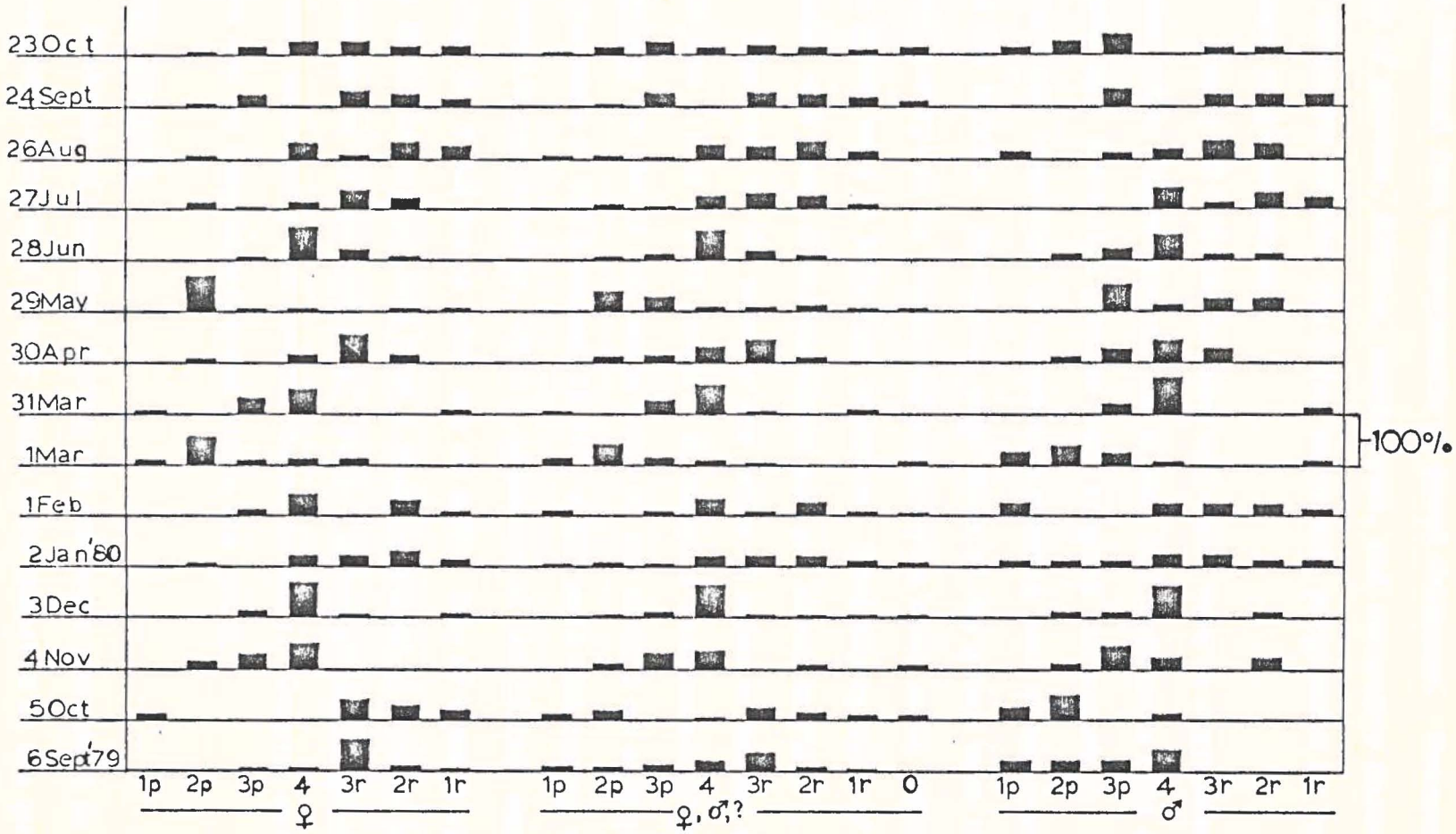

Figure 3. Percentage distribution over time of gonad condition (abscissa) in female, male, and combined sex categories (includes stage 0 - indistinguishable sex) of oysters used for the histological studies.

with the mean gonad index (Figure 4). The mean gonad index can be obtained from measurements of gonad thickness. However, examination of gametes, follicles, amoebocytes, and connective tissue is required to determine whether the index represents progressive or regressive stages of gametogenesis.

Nearly all individuals observed which were in regressive gametogenic stages appeared as if they had undergone only a partial spawning. Some ripe gametes were retained and resorbed. In many individuals resorption of unspawned gametes were widespread throughout the follicles. In others, some areas of the gonad were undergoing resorption by phagocytic amoebocytes while other follicles in the gonad were in fully ripe condition. Photomicrographs of male, female, and those of indeterminant sex are shown in Plate 1.

The mean of the monthly sex ratios of males to females in the field was 1:1.95 ± 0.75 (n=15). Histological examination of Saccostrea cucullata for the 11 full moons prior to the GORCO oil spill produced 2 specimens of 260 examined (0.77%) which were undergoing sex-reversal/hermaphrodism, while 3 specimens of 108 (2.78%) were found during the 4 full moons after the oil spill. One of these 3 specimens was found in August 1980 from a small sample of 12 oysters collected in the center of the spill area. Plate 2 shows photomicrographs of oysters undergoing sex-reversal/hermaphrodism. Oocytes and spermatozoa can be seen interspersed within the gonad.

Lunar Spawning

Evidence of spawning during the full moon was found in histological sections. Ingested ova or sperm were observed in the guts of

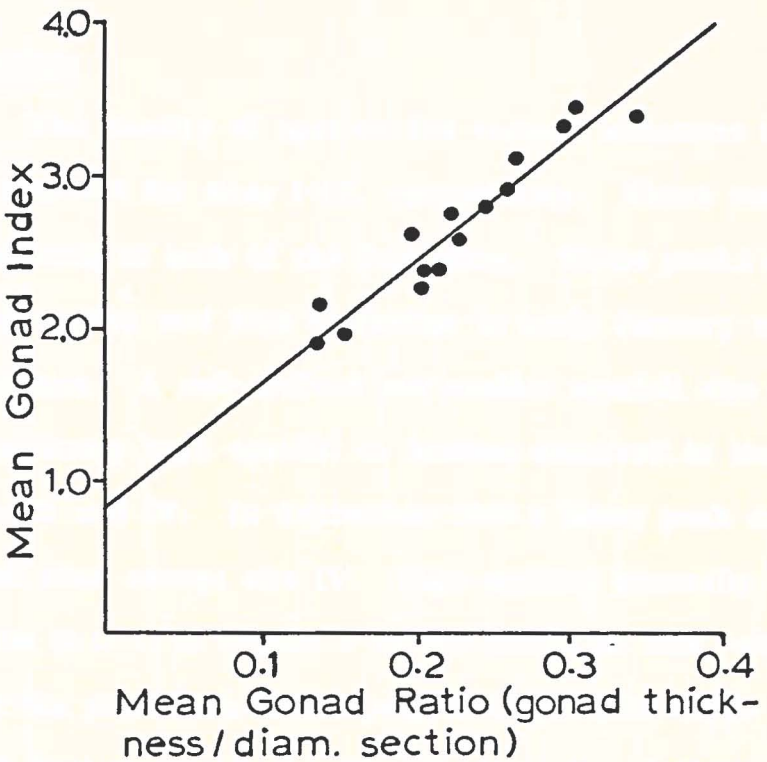

Figure 4. Correlation between mean gonad ratio and mean gonad index for histological sections of Saccostrea cucullata. The mean gonad index is plotted against mean gonad ratio (gonad thickness/diameter of the section). $y=8.004x + 0.838$

oysters in September (n=1), November (1), and December (2) of 1979, and in February (1), June (1), July (1), August (2), and October (1) of 1980. Very recent partial spawnings in oysters were evidenced by empty follicles which had not yet collapsed. However, a significant lunar periodicity of spawning activity was not evident from histological sections.

Spatfalls

The density of spat on the various collectors is shown in Figures 5-8 for sites I-IV, respectively. There were two major peaks of spatfall at each of the four sites. These peaks occurred from April to May and from November to early January on most types of collectors. A well-defined but smaller spatfall also occurred in July. A relatively high spatfall on bamboo occurred in March-April 1980 at sites II and IV. In September 1980 a minor peak in spatfall occurred at all sites except site IV. High spatfall generally followed the peaks of the M.G.I. in Figure 2 by one month or less, which indicates that the planktonic larval periods lasts about three or four weeks. Spat first appeared on collectors at a size of about 0.45 mm. The largest spat observed on collectors were 2-3 mm.

The magnitude of spatfall was consistently greatest at sites I and II. The least spatfall was observed at site III, which was located the greatest distance from both the secondary lagoons and from the main channels in Sasa Bay. A one-way analysis of variance was used to compare peak spatfall on three types of collectors (bamboo, plastic roofing-large corrugations, and plastic roofing-small corrugations). The data were log-transformed because of the great variation in

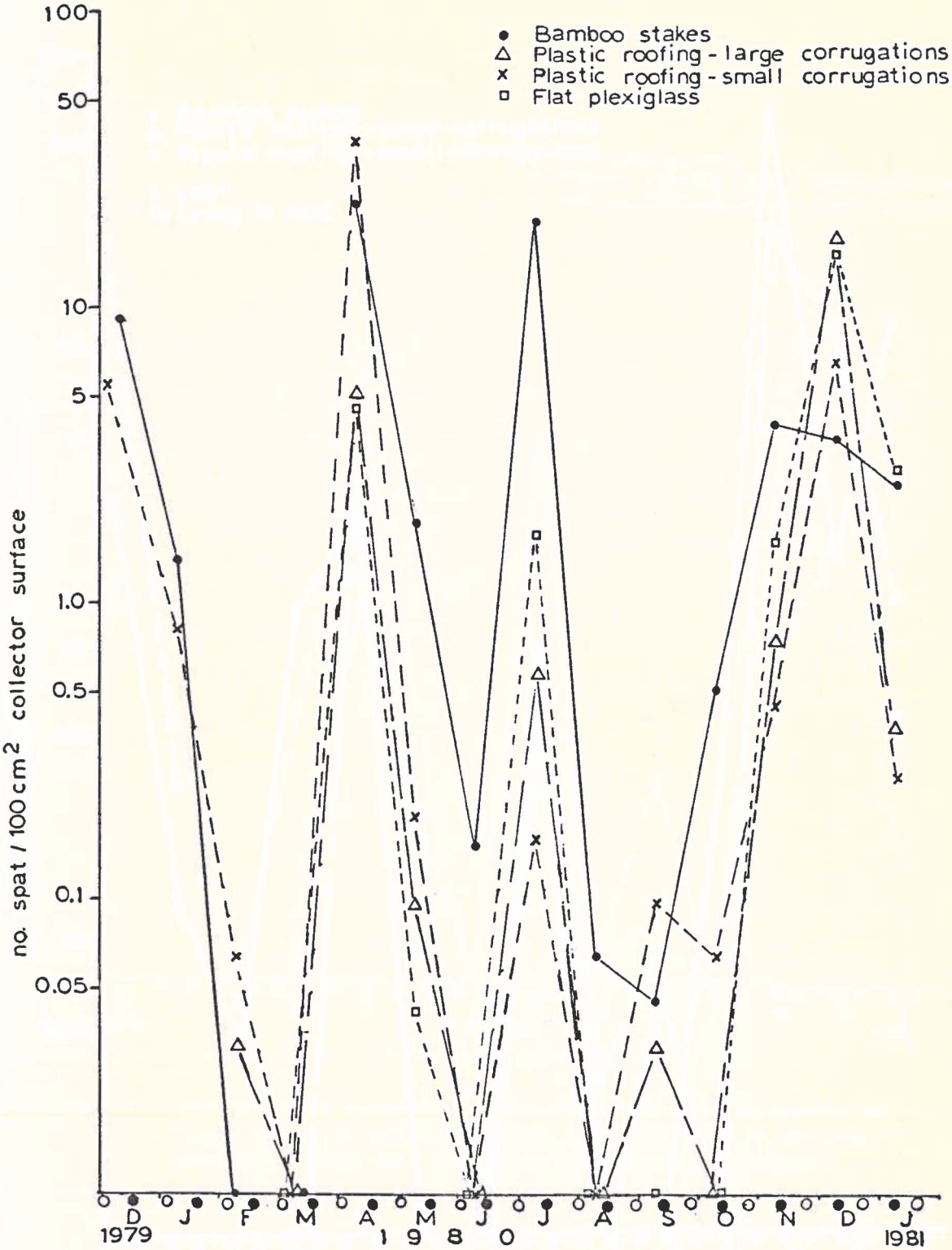

Figure 5. Site I spat collection. Spatfall over time is shown for six types of collectors (no. spat/100 cm^2 of collector surface). Full and new moon phases are indicated.

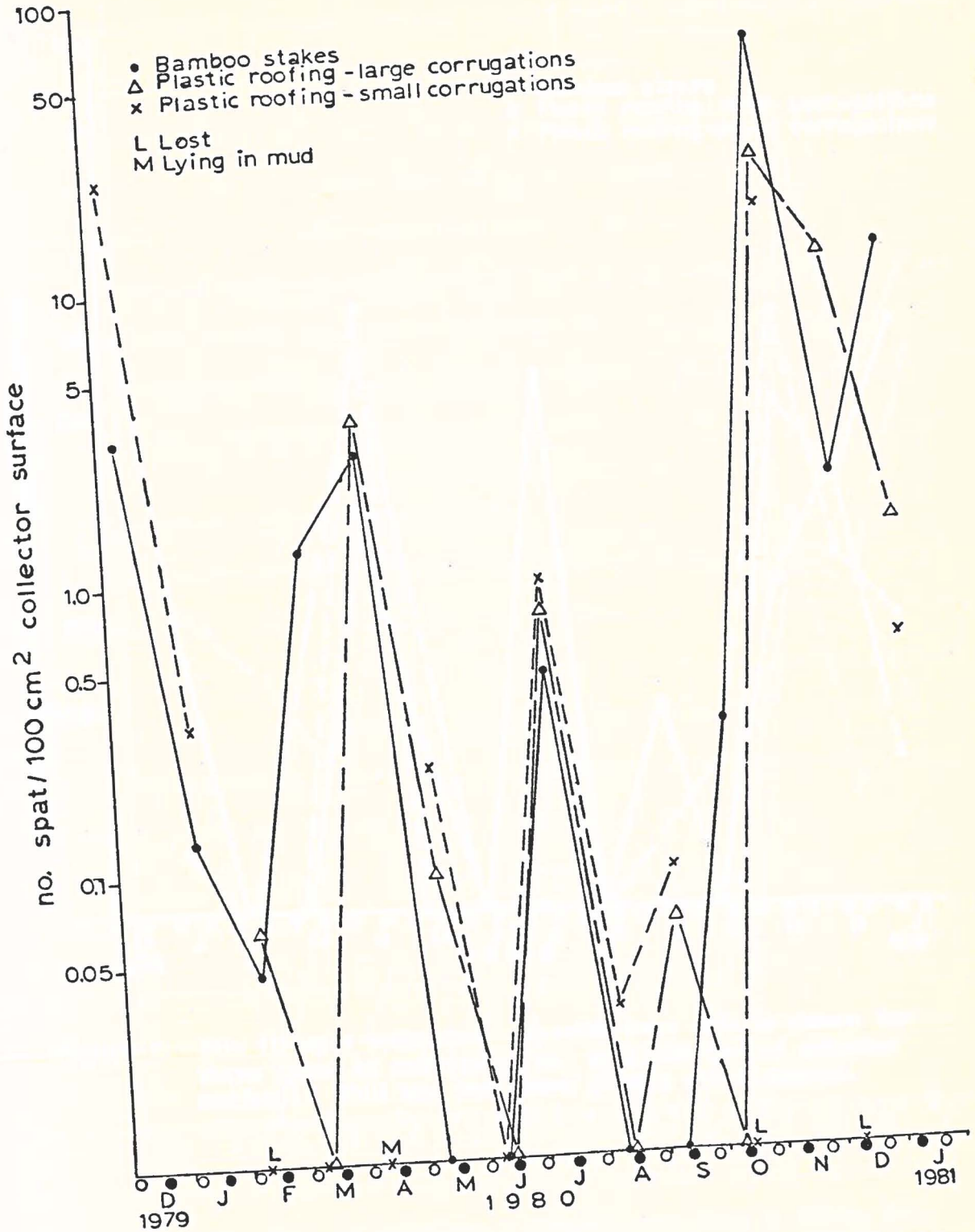

Figure 6. Site II spat collection. Spatfall over time is shown for four types of collectors (no. spat/100 cm^2 of collector surface). Full and new moon phases are indicated.

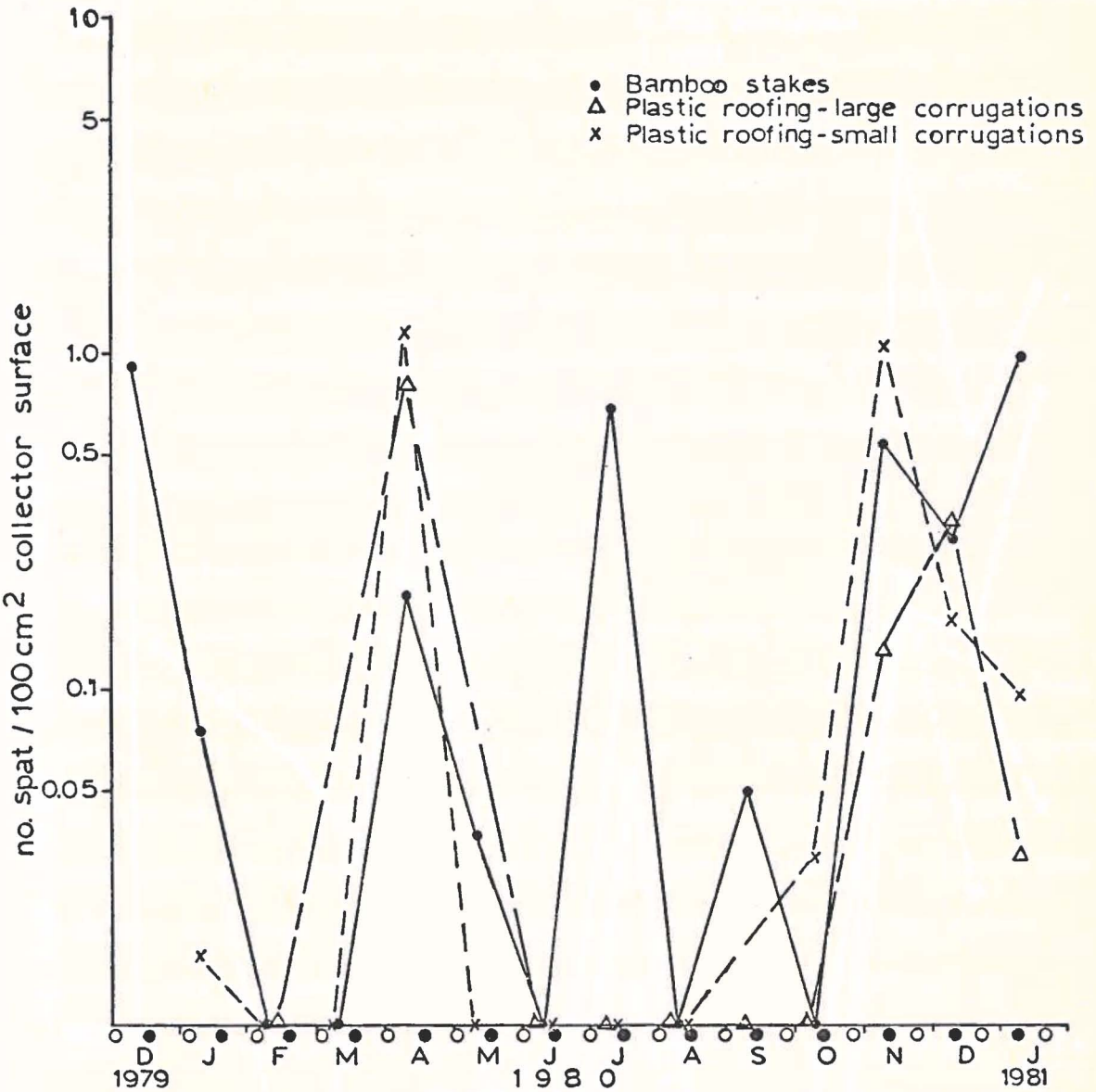

Figure 7. Site III spat collection. Spatfall over time is shown for three types of collectors (no. spat/100 cm^2 of collector surface). Full and new moon phases are indicated.

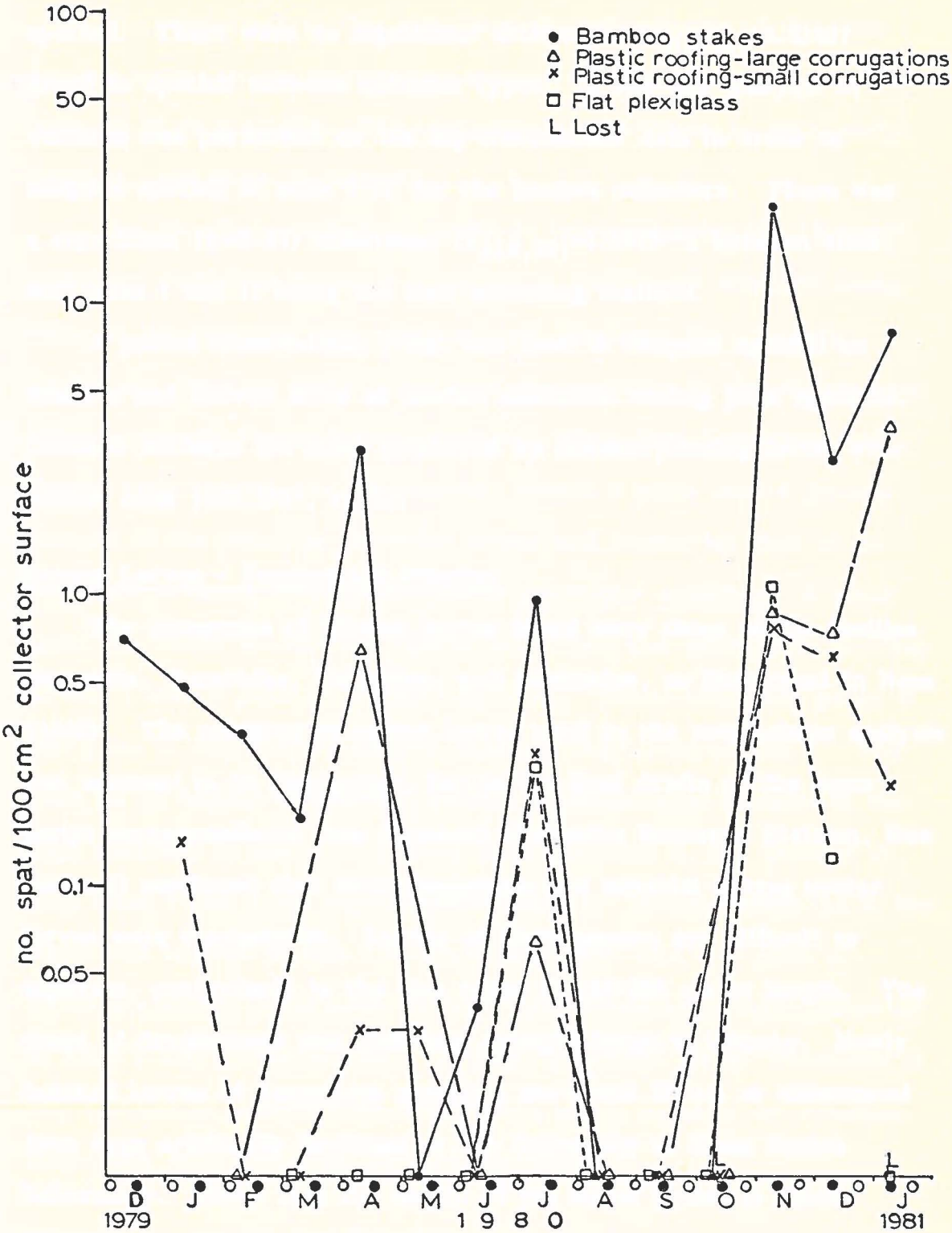

Figure 8. Site IV spat collection. Spatfall over time is shown for four types of collectors (no. spat/100 cm^2 of collector surface). Full and new moon phases are indicated.

spatfall. There were no significant differences ($F_{s(2,57)}=2.8189$) found in spatfall between collector types. A one-way analysis of variance was performed on the log-transformed data in order to compare spatfall at sites I-IV for the bamboo collectors. There was a significant ($p<0.01$) difference ($F_{s(3,20)}=5.5876^{**}$) between sites, with sites I and II being the best collecting stations.

A paired observations t-test was used to compare spatfall on concave and convex sides of bamboo collectors during peak spatfalls. Concave surfaces are much preferred by metamorphosing larvae over convex ones ($t=2.564^{*}$, $v=20$).

Oyster Larvae - Zooplankton Samples

The categories of bivalve larvae found were those of the families Ostreidae, Veneridae, Tellinidae, and Pectinidae, as illustrated in Rees (1950). The category of oyster larvae used in the zooplankton analysis was assumed to be Saccostrea, but in the final drafts of the manuscript correspondence from Dinamani (Fisheries Research Division, New Zealand) confirmed that both Saccostrea and subtidal Ostrea oyster larvae were represented. Larvae of these genera are difficult to separate, particularly in the size range of 150-200 µm in length. The stage of development was from late straight hinge to umbone. Newly hatched larvae of Saccostrea cucullata are about 50 µm as determined from eggs artificially fertilized at the University of Guam Marine Laboratory. Given that the length of the larval period is about three or four weeks and that spat appear at 0.45 mm, an age of 6-10 days was estimated for this size class by assuming linear growth for this short period of time.

The percentage of this size class of oyster larvae of the total bivalve larvae in the zooplankton samples ranged from 6-80%, with a mean of 38.62 ± 21.64%. The proportions were lowest in July, early October, and latter December.

The abundance of oyster larvae in the water over the 13-month period is plotted in Figure 9. The mean counts and standard deviations of the replicate tows taken at each site over the 13-month period are listed in Table 1, to show the variation in the counts from which values in Figure 9 were derived. Since both Saccostrea and Ostrea type oysters are represented, Figure 9 gives only a general pattern of the presence of oyster larvae and cannot be used as an indicator of timing of reproduction in Saccostrea cucullata. A major peak was found in mid-May 1980 at site 2 and was followed in late May at site 1 with a peak of similar magnitude. Other noticeable peaks at site 1 on 11 July and 12 August were not duplicated at site 2. The peak on 12 August at site 1 is somewhat questionable because of the high variation in counts. A major peak occurred in late October at both sites 1 and 2 but was greater at site 1. Density of larvae in the water was highest at site 3 in early November, and this peak of larval abundance progressed through late November to early December at sites 2 and 1, respectfully. Finally, a peak in larval abundance at site 2 occurred in early February, while site 1 remained at a medium abundance from December through February.

Physical Parameters and Tidal Flushing

Water surface temperatures taken at zooplankton sites 1 and 2 are plotted in Figure 10. Little variation is evident at any time.

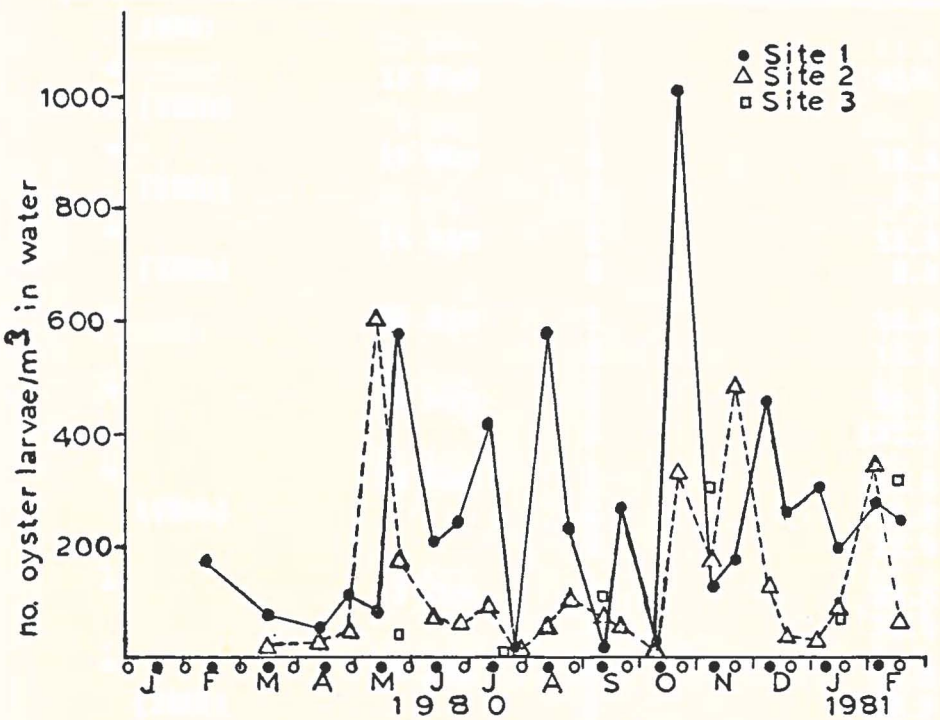

Figure 9. Abundance of oyster larvae (no. larvae/m^3 in water) as shown through time for three sites in Sasa Bay.

Table 1. Mean counts of oyster larvae in tow samples; 1/32 of sample counted. To determine no. larvae/m^3 in water multiply mean values: x 32 ÷ 9.6359 m^3(2.5 min. tows); *for 12 February and 19 March 1980 x 32 ÷ 7.7087 m^3(2.0 min. tows). Date of full or new moon is date of sampling unless indicated in parenthesis.

Moon	Date	Site	Mean tow 1 & 2 + s.d.
1980:			
• (16th)	12 Feb	1	41*
• (16th)	19 Mar	1	16.5 ± 7.1*
		2	4.8 ± 0.4*
• (15th)	14 Apr	1	12.8 ± 6.0
		2	8.0 ± 7.1
°	30 Apr	1	35.0 ± 7.8
		2	13.2 ± 6.0
•	14 May	1	24.2 ± 5.3
		2	181.2 ± 62.6
° (29th)	26 May	1	114.0 ± 68.6
		2	51.0 ± 33.2
		3	12.0 ± 4.9
•	12 Jun	1	62.0 ± 41.7
		2	21.5 ± 16.9
° (28th)	27 Jun	1	73.0 ± 29.0
		2	17.5 ± 5.6
• (12th)	11 Jul	1	124.0 ± 37.5
		2	28.0 ± 2.1
°	27 Jul	1	4.8 ± 1.1
		2	0.2 ± 0.4
		3	0.5 ± 0.7
• (10th)	12 Aug	1	174.0 ± 206.5
		2	7.2 ± 3.9
°	26 Aug	1	67.8 ± 27.2
		2	30.8 ± 15.9
• (9th)	11 Sept	1	5.8 ± 1.8
		2	20.8 ± 22.3
		3	33.0 ± 16.9
°	24 Sept	1	80.0 ± 6.4
		2	15.5 ± 7.1
•	9 Oct	1	7.2 ± 1.8
		2	2.8 ± 1.1

Table 1 (Continued).

Moon	Date	Site	Mean tow 1 & 2 + s.d.
○	23 Oct	1	304.0 ± 136.5
		2	99.2 ± 61.9
●	7 Nov	1	36.2 ± 1.8
		2	51.8 ± 64.7
○	24 Nov	1	53.2 ± 14.5
(22nd)		2	146.5 ± 31.8
●	7 Dec	1	137.8 ± 20.2
		2	36.2 ± .1
○	21 Dec	1	77.5 ± 12.0
		2	11.2 ± 5.3
1981:			
●	5 Jan	1	92.0 ± 27.6
		2	9.5 ± 7.8
○	19 Jan	1	59.2 ± 3.9
		2	27.0 ± 14.1
		3	21.2 ± 4.6
●	5 Feb	1	82.0 ± 2.1
		2	102.8 ± 11.7
○	19 Feb	1	76.5
		2	18.5 ± 7.8
		3	95.5 ± 9.8

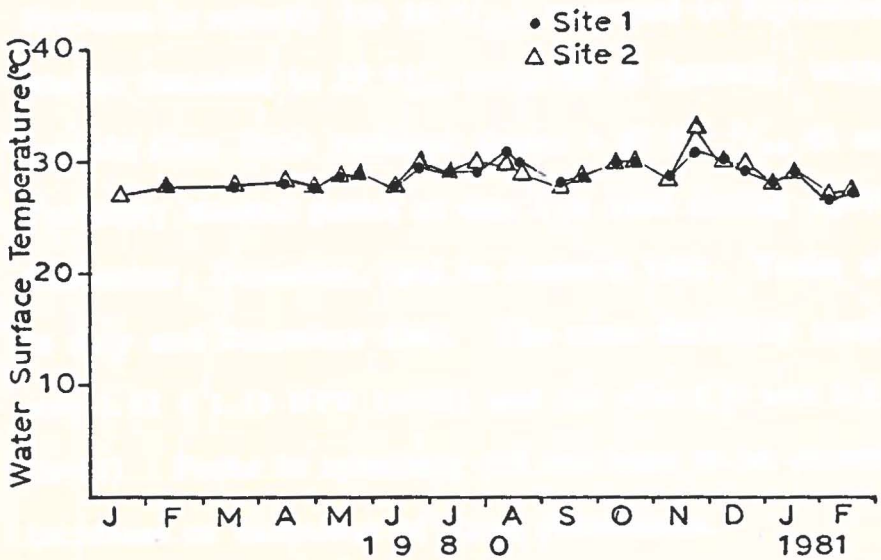

Figure 10. Water surface temperature taken with time at two zooplankton tow sites in Sasa Bay.

Figure 11 shows the total precipitation per month and the percent of possible sunshine over the same time period. Precipitation peaked in February and September, with a consequent decrease in percent of possible sunshine.

Salinity and turbidity are shown in Figure 12. The greatest decrease in salinity (to 16 $^{\circ}/_{oo}$) occurred in September 1980. A smaller decrease to 27 $^{\circ}/_{oo}$ occurred in October, while all other periods show little variation (mean = 33 ± 4 $^{\circ}/_{oo}$ at each site). Turbidity showed peaks at site 1 in 1980 during April, May, July, September, December, and in January 1981. Peaks at site 2 were in July and December 1980. The mean turbidity reading for site 1 was 2.22 ± 1.19 NTU (n=26) and for site 2 it was 2.13 ± 1.86 NTU (n=27). Peaks in spawning did not seem to be correlated with increases or decreases of these parameters.

Slow tidal flushing in Sasa Bay was indicated from the low rates of movement of 1-m cloth drogues. A maximum rate of about 0.1 m/sec was recorded about two hours before low tide while a minimum rate of 0.002 m/sec was recorded shortly after low tide. Wind speed was light (range 0.5-3.5 m/sec) from NE 310-330°. Figure 1 shows arrows representing the direction the drogues moved at the six stations sampled. The hypothesis that larvae produced by the population of oysters in Sasa Bay are retained within this bay is supported with this evidence of slow tidal flushing.

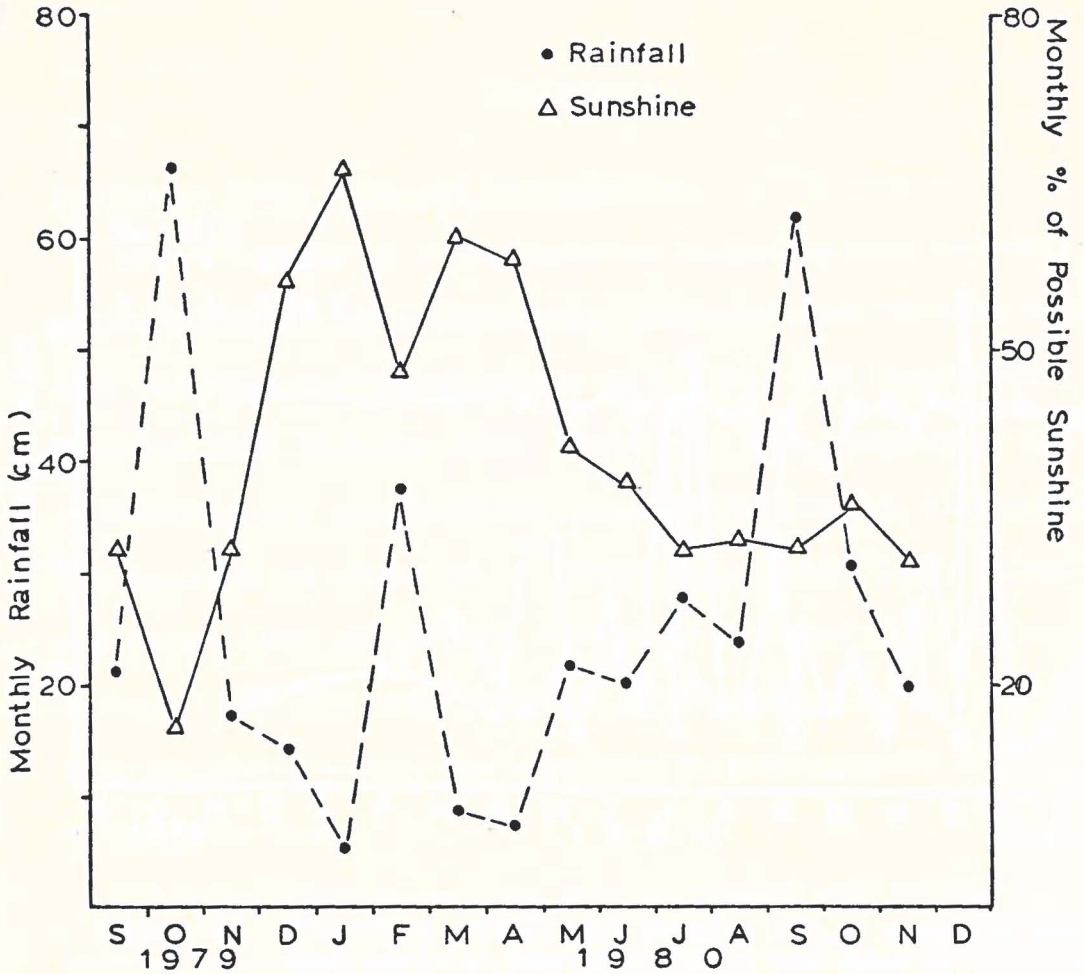

Figure 11. Local climatological data: monthly rainfall in cm, and monthly percent of possible sunshine during daylight hours. Data from Guam, Pacific Nat. Weather Serv. (NOAA).

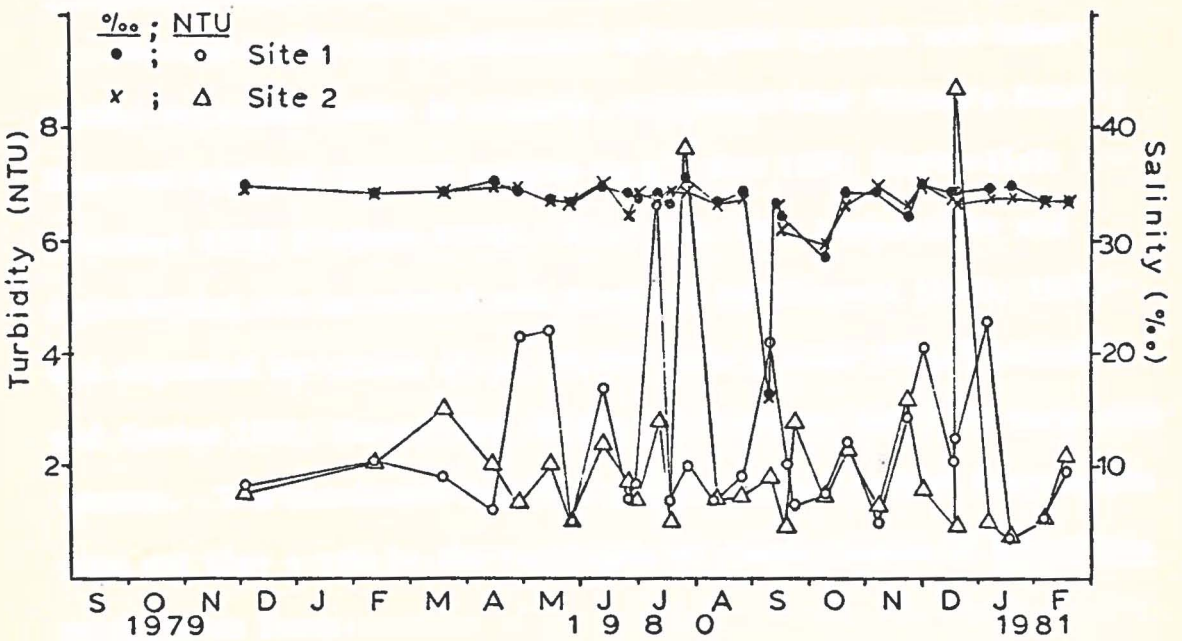

Figure 12. Turbidity (NTU) and Salinity ($^{\circ}/_{oo}$) readings over time taken at two zooplankton tow sites in Sasa Bay.

DISCUSSION

Exogenous Clues Affecting Reproduction

Several works on reproduction of tropical oysters and other marine organisms have, in essence, suggested that "Orton's Rule" does not apply to tropical species (e.g., Rao 1956; Durve 1965; Johannes 1978; Stephen 1980). This rule, that temperature and reproduction are correlated was proposed by Thorson (1946, 1950) and espoused by others (e.g., Orton 1920; Nelson 1928; Loosanoff and Davies 1952; Hasan 1964; Wilson and Hodgkin 1967) for temperate species. The conclusion from the present report is that temperature does not play a role in the regulation of gametogenesis of Saccostrea cucullata on Guam.

Salinity fluctuations have been correlated with spawning in tropical oysters (Rao 1956; Giese and Pearse 1974; Stephen 1980) in areas where heavy monsoonal rains occur. Stephen (1980) suggested calling this correlation "Hornell's Rule" after the author who first proposed the relationship. A salinity of 20-25 $^{\circ}/_{oo}$ was found to be associated with the major spawning of Crassostrea madrasensis (Stephen 1980). However, fluctuations in salinity were small in Sasa Bay, which had a moderately high 'estuarine' salinity (33 $^{\circ}/_{oo}$). This is similar to salinities found in Singapore (range: 28.64-31.08 $^{\circ}/_{oo}$) by Ling (1970) and those found in East Africa (range: 28-40 $^{\circ}/_{oo}$) by Van Someren and Whitehead (1961) for areas with populations of S. cucullata.

The lack of correlation between lunar periodicity and major spawning activity for Saccostrea cucullata in Sasa Bay was supported by findings of Van Someren and Whitehead (1961) for this species in East Africa. Korringa (1947) and Orton (1926) found that spawning of Ostrea edulis was correlated with new and full moons. This could reflect a combination of exogenous factors (temperature and moon phase) acting together during the relatively short summer breeding season in higher latitudes. The sampling methods used in this study may have masked evidence of lunar periodicity.

Glycogen stores increase in oysters before and during gametogenesis (Galtsoff 1964) and Giese and Pearse (1974) speculated that fluctuations in food supplies affect nutrient reserves to which gametogenesis is sensitive. Crisp and Davies (1955) showed a relationship between food abundance and reproduction in the barnacle Elminius modestus. The population of S. cucullata in Sasa Bay moves rapidly from one gametogenic cycle to the next with little 'resting' and concomitant build up of nutrient reserves. The level of food supply in Sasa Bay must be stable in order to provide the energy required for this continuous production of gametes. Stephen (personal communication) has found many small peaks in spawning and a relative lack of definite resting stages in tropical oysters and mussels from coastal India.

With a condition of continuous spawning in Saccostrea cucullata populations in Sasa Bay and a lack of any apparent exogenous clues to spawning, it is difficult to know exactly how many gametogenic cycles are covered annually by individuals. There may be more gametogenic cycles of the population as a whole than for an individual.

Moderate or poor synchronization in spawning would be evidence for a lack of exogenous clues for spawning (Stephen, personal communication; Giese and Pearse 1974).

Partial Spawning and Resorption of Gametes

Partial spawning and resorption of unspawned gametes were commonly found in this study. Moore (1972) speculated that because of the energy lost by phagocytosis of unspawned sex products, it would be advantageous for individuals in the population to synchronize spawning as closely as possible. Why then the apparent waste in producing so many gametes only to resorb many of them? Perhaps this may be a result of stress on spawning in Saccostrea cucullata. It has been shown that spawning in some tropical oysters (Crassostrea gryphoides and C. madrasensis) will not occur without a decrease in salinity (Durve 1965) although gametogenesis still occurs (Stephen 1980). Under hypersaline conditions (>35 $^{\circ}/_{oo}$) resorption of gametes takes place (Durve 1965). Partial spawning may be related to the relatively high mean salinity of 33 $^{\circ}/_{oo}$ in Sasa Bay. However, Stephen (personal communication) says that it is usual to find a fairly large number of eggs left in spent oysters. These remaining eggs may be immature as a result of poor synchronization of spawning, which in turn may result from a lack of exogenous clues in a stable environment (Stephen, in press).

Oil Spill and Sex-Reversal/Hermaphrodism

It is interesting that at zooplankton site 2 (near the oil spill site) the abundance of oyster larvae was low for nearly five months after the spill, while higher peaks occurred at site 1 during this time.

Moore (1958) noted that oysters which are not killed directly by pollutants may be harmed by remaining closed for long periods. Prolonged closure would hamper normal feeding and result in poor condition. Barszcz et al. (1978) made histological observations on oysters exposed to single low level (4 ppm) doses of three crude oils. Condition of the oysters was poor after exposure and a reduction in the development of the germinal epithelial tissues indicated reduced reproductive potential. In this study a higher incidence of sex-reversal/hermaphrodism occurred (2.78%) during the post-spill period than in the pre-spill period (0.77%). Sex-reversal/hermaphrodism is relatively rare in Crassostrea [and Saccostrea] (Galtsoff 1964). However, it has been shown that the sex may be reversed after spawning occurs (Needler 1932, 1942; Galtsoff 1937, 1964). Stressed environments or fluctuating environmental factors may influence the sex changes of oysters (Coe 1932, 1934; Rao 1956).

Cultivation

Sasa Bay appears to be an ideal site for the culture of bivalves. The Australian method of rack culture (Bardach et al. 1972; Quayle 1980) would be relatively easy to adapt to the physiographic conditions in the bay. The numerous reef platforms are mostly exposed at minus tides, which makes them attractive for off-bottom culture. Many hectares of reef platform would be available for this type of cultivation. Nicolic et al. (1976) describe a similar system for farming Crassostrea rhizophorae in Cuba.

Bamboo has been used extensively in the Philippines, Taiwan, and other tropical countries for spat collection and cultivation (Ablan

1949; Blanco et al. 1951; Bardach et al. 1972). There is potential for setting up large spat collection stations near sites I and II where cultch material, such as local bamboo, wood stakes or manufactured materials, could be used. These collectors with spat could then be transferred to racks for commercial grow out. Data from this study indicate that spat collectors providing as many concave faces as possible would be the most efficient. Thomson (1950) noted that studies have shown setting by oyster larvae require at least relatively slack water, thus higher spat collection was found inside closely set slats. A concave surface also tends to slow water movement. An ideal collector could be made by tying three split bamboo stakes together with the concave sides facing outward, thus the convex sides would be protected and settling space maximized.

Experimental raft culture in Cuba with the mangrove oyster Crassostrea rhizoporae has produced 6,600 kg; ha^{-1} per year (Bardach et al. 1972). Secondary lagoons in Sasa Bay could be utilized for raft culture with rack culture on reef platforms. Bamboo stake culture, racks, and racks with strings of spatted shells are among methods of cultivation used in the Philippines, where production may be 3.82 metric tons whole oyster (1.06 metric tons meat only) per 2500 m^2 plot/yr (Guerrero et al. 1977). C. iredalei and C. malabonensis are the primary cultivated species, but Saccostrea cucullata is sometimes farmed. One negative feature of S. cucullata as a mariculture species is its small size. However, in some Southeast Asian countries such as Singapore, the sweeter taste of this oyster is preferred to that of larger species (Ling 1970).

Sasa Bay is the only area on Guam which supports a dense population of intertidal oysters. Spawning and spatfall occur year around at a low level, with several major peak periods. Thus, newly settled juveniles could be obtained on a regular basis for mariculture. Sasa Bay may also be used for collection of spat, and the cultch could then be moved to other areas where the grow out could be accomplished. This practice is common for mussels and oysters in Australia, New Zealand, and several countries in the northern hemisphere (Bardach et al. 1972). Should Sasa Bay be zoned for mariculture by the Government of Guam, shellfish culture would be possible in this locality.

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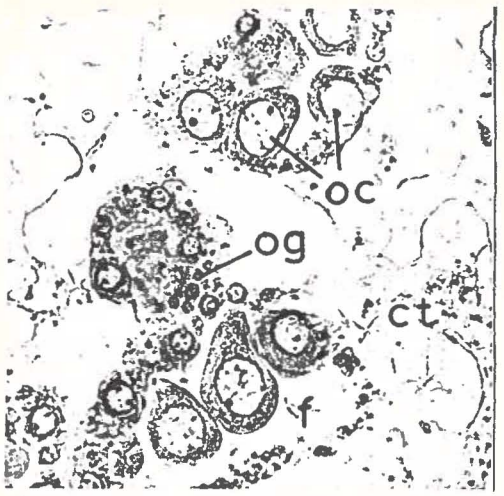
PLATE 1: Photomicrographs of histological sections of Saccostrea cucullata showing stages of ripeness for males, females, and those of indistinguishable sex. A-G, female; H-N, male; O, indeterminant sex, resting. A. Stage 1p; oogenesis commences. Oogonia (og) and oocytes (oc) shown with connective tissue (ct) surrounding follicles (f). 200x. B. Stage 2p; 1/2 ripe ovary. 40x. C. Stage 3p; 2/3 ripe ovary with mostly ripe oocytes. 40x. D. Stage 4; fully ripe ovary with numerous ova packed in polygonal formation (po). Connective tissue reduced. 40x. E. Stage 3r; post spawning, partially emptied gonad. 100x. F. Stage 2r; more advanced regressive stage. Some residual ova (ro) being cytolyzed. 100x. G. Stage 1r; very advanced regressive stage. Few residual ova remain in contracted follicles and connective tissue increases. 40x. H. Stage 1p; spermatogenesis begins. Digestive diverticula (dd) shown. 40x. I. Stage 2p; male. Intestinal fold (i) with diatoms inside is shown. 40x. J. Stage 3p; male. 40x. K. Stage 4; fully ripe testis. Spermatogonia and spermatocyte (spg & spc) stages reduced while ripe spermatozoa (spz) packs the testis. 40x. L. Stage 3r; male. Phagocytic amoebocytes #1 (ph 1) and #2 (ph 2) shown along follicle walls cytolyzing residual sperm. 100x. M. Stage 2r; male. Increase in connective tissue and cytolysis of unspawned spermatozoa continues. 100x. N. Stage 1r; male. 40x. O. Stage O; indeterminant sex, resting. 100x.



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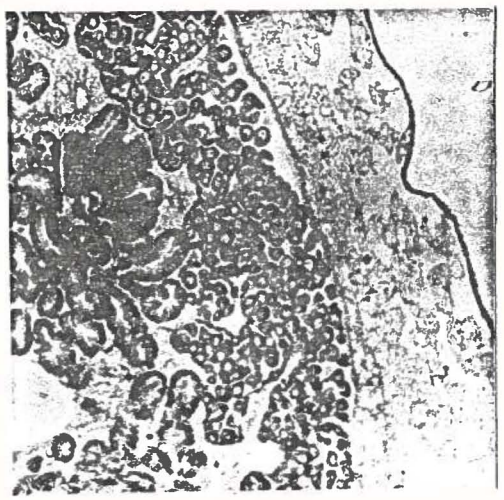

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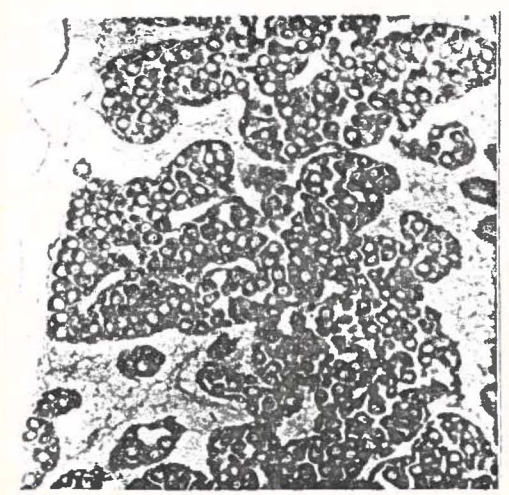

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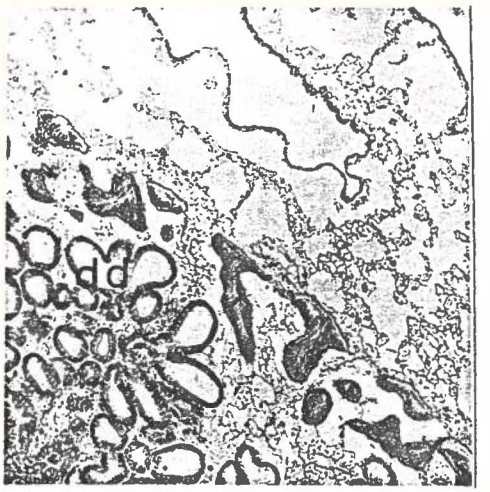

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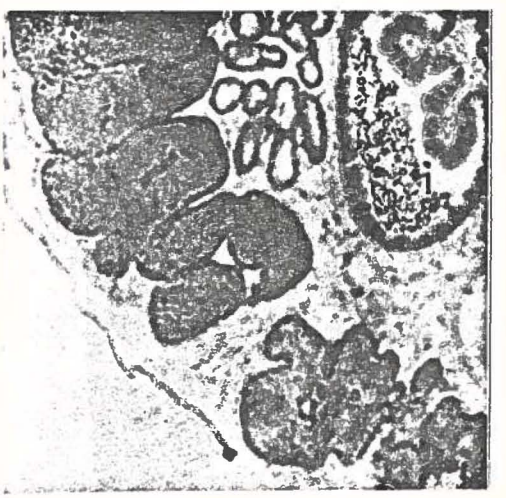

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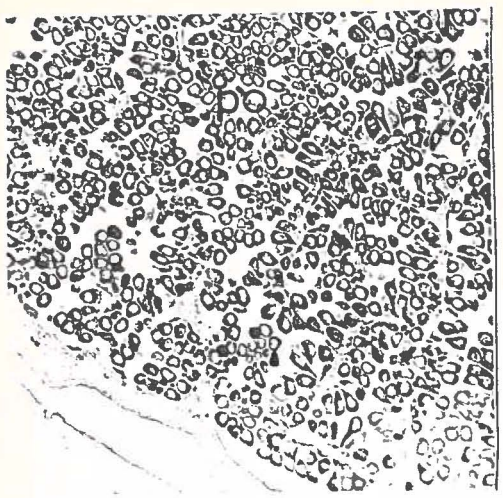

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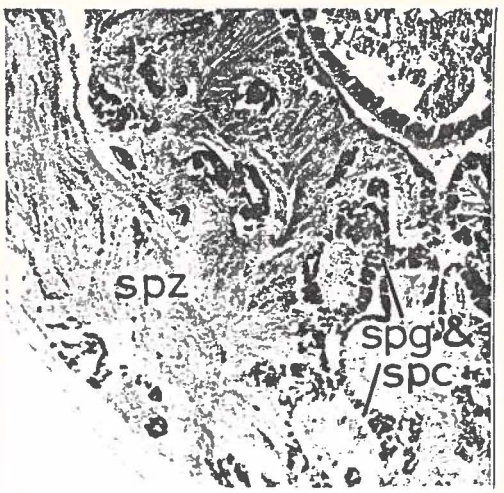

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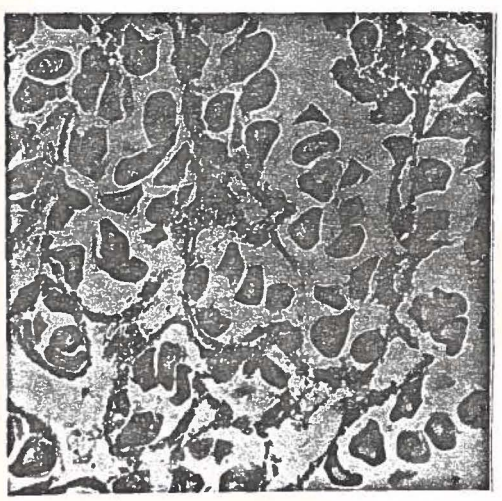

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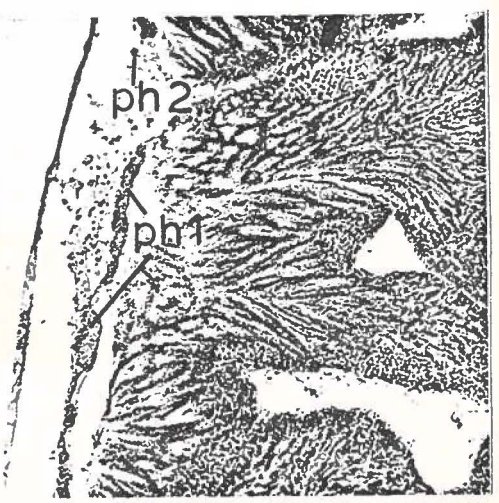

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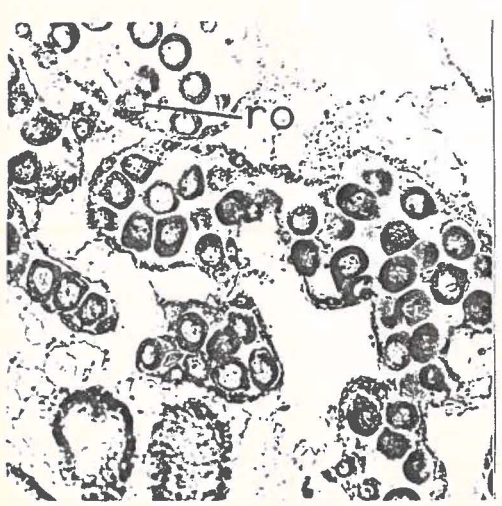

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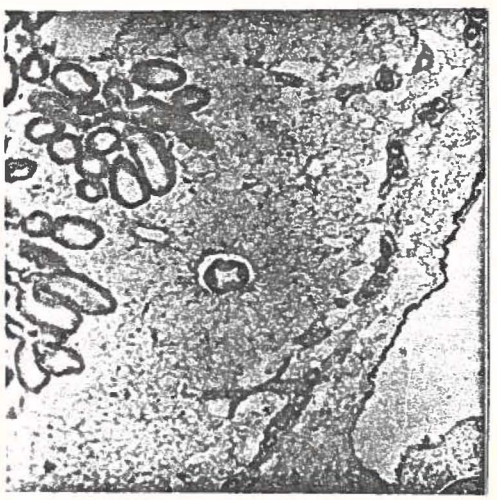

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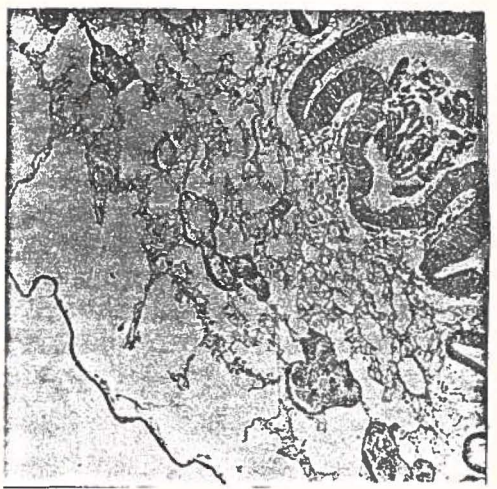

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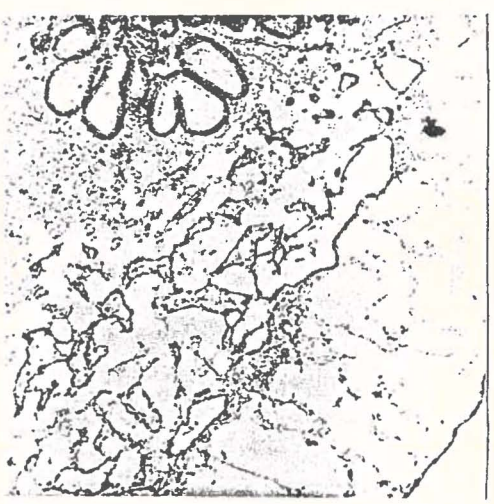

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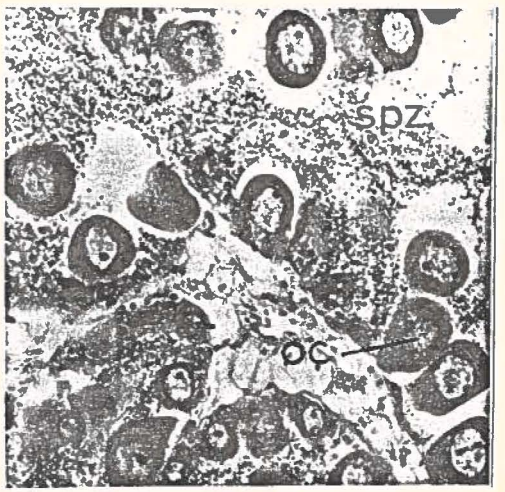
PLATE 2: Photomicrographs of histological sections of Saccostrea cucullata showing sex-reversal/hermaphrodism. A. July 1980. Spermatozoa (spz) and oocytes (oc) interspersed throughout gonad. B. September 1980 specimen similar to A. C. 31 March 1980. Residual sperm and ova in gonad with #2 phagocytic amoebocytes (ph 2) shown along follicle wall (fw) and connective tissue (ct). D. April 1980. Primarily male gametes with a few primary oocytes clustered throughout the gonad. E. August 1980. Primarily male gonad. Note cluster of primary female sex cells surrounded by connective tissue. All photos are 200x.



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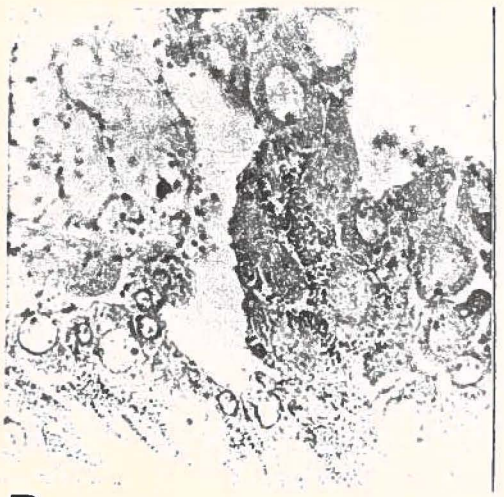

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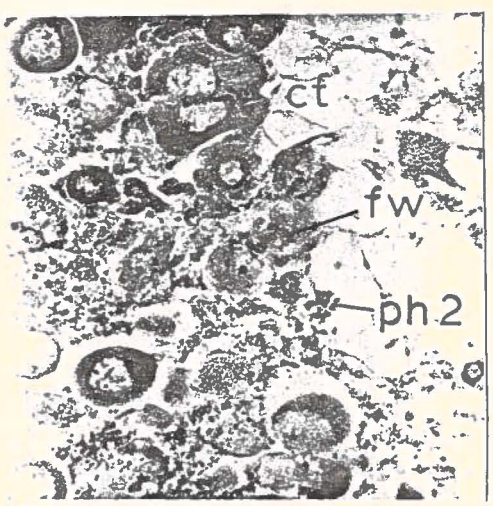

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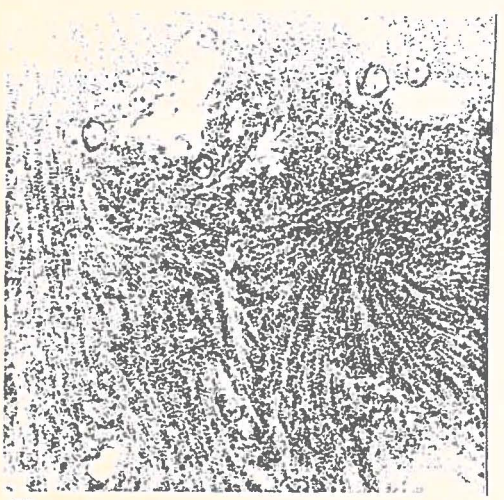

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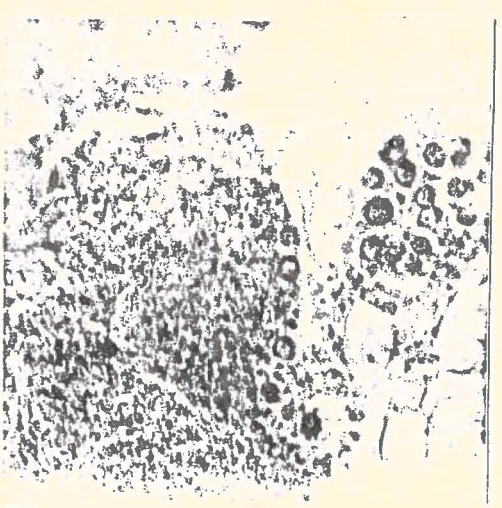

E