REPRODUCTIVE BIOLOGY OF THE TROPICAL CLAM *DONAX DENTICULATUS* IN EASTERN VENEZUELA

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ABSTRACT

The reproductive biology of the tropical clam *Donax denticulatus*, an important Venezuelan fishery resource, was studied for 24 months. Histological examinations were made of gonadal tissue from 966 clams collected from a population on the coast of the Araya Peninsula. Gametogenesis and sexual differentiation began asynchronously when juveniles were approximately 9 mm long. Half of the juveniles spawned for the first time at 6-7 months. Adults clams reproduced continuously throughout the year, but showed greatest activity between July and December, when water temperatures were highest (28°C). Sex ratio was 1:1 in all samples of individuals longer than 9 mm.

INTRODUCTION AND METHODS

Punta Araya beach, located on the Araya Peninsula shoreline in eastern Venezuela (Fig. 1), was selected for this study. It holds one of the largest populations of *D. denticulatus* in eastern Venezuela. Salinity of the water close to the study area showed no marked variation (36.5-36.8 °/oo) during the investigation period. Previously, Gade (1961, a&b) reported annual salinity values between 36.3 °/oo and 37.0 °/oo for the same environment. The seasonal variation of Orinoco River water does not affect the salinity of the northern Venezuelan Coast (Gade, 1961c). The entire tidal variation lies within the range of 50 cm (Gade, 1961a).

The diurnal variation of interstitial water temperature of *D. denticulatus* habit was measured from June 1974 to May 1976 (Fig. 2). The highest temperatures (27 and 29°C) were recorded between July and November, and the lowest temperatures (22 to 25°C) from January to May.

D. denticulatus were collected during 24 consecutive months (June, 1974 to May, 1976). Representative samples of all cohorts in the population were taken from four transects located at right angles to the beach, based on the dispersion pattern established for *D. denticulatus* (Wade, 1967). The material from each transect was passed through four sieves with mesh sizes of 5, 2, 1 and 0.5 mm, respectively. Samples retained by the first two sieves were counted directly; while those retained by the finer sieves were counted by aliquot subsamples. Counting and measuring small juveniles were aided by treatment of subsamples with Rhodamine B and observation in a Bogorov Camera Under a dissecting microscope.

Gametogenesis, maturation and sex ratio were examined histologically in individuals collected during 16 months. The monthly samples selected for histological examination were fixed in Davidson's solution for 48 h, then preserved in a mixture of glycerine and 70% ethanol (1:9 VVV). Paraffin em-

Carib. J. Sci. 21(3-4). 125-136 (1985)

bedded transverse sections (7 µm) were taken from the area immediately posterior to the labial palps and included viscera, gonads, gills and digestive diverticula. The sections were stained with Delafield's haematoxylin, then counterstained with eosin using standard procedures. The reproductive condition of cells within different regions of the gonad varied. The reproductive status of the sample was therefore determined by dividing each gonad section into ten sub-areas and determining the sexual condition of the cells within these areas. The frequency of each reproductive stage was then based on the 10 observations of 35 clams, or a total of 350 observations.

The size, sex ratio, and maturity were studied by histological examination of 966 individuals having antero-posterior shell lengths of 5 to 24 mm; this included most size classes. The annual reproductive cycle was estimated by the histological examination of 35 adult specimens per month and the observation of recruitment of spats. The pattern of probability curves (Wenner, 1972) and the hypothetical relation of 1:1 (Simpson, 1967) were used to establish the nature of changes in the sex ratio.

The reproductive cycle of female *D. denticulates* was arbitrarily divided into five stages following the general pattern described by Porter (1964), Ropes (1968), Keck *et al.* (1975), Vélez (1977), and Andrade and Lunetta (1978) with modifications for the species concerned as follows:

The *Early Active Stage* (EAS) is characterized by presence of small follicles surrounded by abundant connective tissue (Fig. 3A). In virgin females, the follicles have a small empty lumen, whereas the lumen is occupied by a few residual oocytes in spent females and those restarting gametogenesis. (Fig. 3F).

The *Late Active Stage* (LiS) is characterized by increased size and number of follicles (Fig. 3B). Consequently, the interfollicular connective tissue is considerable reduced. There is increase in the proportion of pear-shaped oocytes, now attached to the follicular wall only by a penduncle. Juvenile females that have passed through the EAS and those that have partially spawned and restarted oogenesis exhibit LiS. In the latter, the ample follicular lumen contains some residual oocytes and the follicular wall is invaded by growing oocytes.

The *Mature Stage* (MS) is characterized by follicles and oocytes which have reached maximum size and number (Fig. 3C). The gonadal connective tissue is reduced to simple interfollicular partitions. Numerous oocytes that have separated from the follicular wall now occupy the lumen.

In the *Partially Spawned Stage* (PS) there are numerous follicles with half-empty lumens (Fig. 3D). The interfollicular connective tissue shows characteristics similar to those described for the previous stage. However, in this stage the interfollicular walls are shrunken and broken, so that the gonadal structure appears disorganized. The gonads reorganize and restart gametogenesis in the LiS (Fig. 3F), or become undifferentiated with loss of vesicular connective tissue (Fig. 3E) in the *Undifferentiated Stage* (US).

The male reproductive cycle was divided into five stages in accordance with patterns described by Porter (1964), Ropes (1968), Keck, *et al.* (1975), Velez (1977) and Andrade and Lunetta (1978) with modifications:

In the Early Active Stage (EAS) the sexual tubes are dispersed in the gonad and have numerous primary gonocytes implanted in their walls (Fig. 4A). There are also concentric bands of spermatocytes, spermatids and spermatozoa converging toward the center of the lumen.

The gametes in the *Late Active Stage* (LiS) grow and mature rather than increase in number. Transverse sections of the "tubo-testis" show that the intertubular connective tissue in considerably reduced in most of the gonadal area (Fig. 4B). As in females, those males that have gone through the EAS, or have partially spawned and restarted gametogenesis demonstrate LiS.

The *Mature Stage* (MS) is characterized by the gonad being almost completely occupied by "tubo testis" (Fig. 4C). Their lumen is filled mainly by spermatozoa converging towards the center. The walls of the testes show no active spermatogenesis, though sometimes a thin band of spermatocytes or spermatids can be observed. The connective tissue has been reduced to simple interfollicular partitions.

Male spawning is not as easy to recognize as in females. The *Partially Spawned Stage* (PS) can be identified by observing the morphology of transverse sections of "tubo-testis" and also the organization and developmental stages of the gametes (Fig. 4D). Transverse sections of the "tubo-testis" are circular during maturity, but become polygonal after spawning due to shrinkage.

RESULTS

Size, Sex, Ratio and Maturation.

Approximately 3% of juveniles with a shell length of 7 to 9 mm showed signs of gametogenesis but sex was difficult to determine in all classes below 11 mm in length. The number of sexually mature individuals increased progressively to the 17-19 mm group, in which 98% could be sexed (Fig. 5). Males and females were equally represented in all juvenile and adult size classes following a standard probability curve and the hypothetical relation of 1:1 males to females (Fig. 6).

Annual Reproductive Cycle

The annual reproductive cycle has been defined as all reproductive cycles occurring in 1 year (Giese and Pearse, 1974). Here the term reproductive, including early differentiation of sex cells, their growth (females), multiplication (males), and release.

The presence of more than one stage of the reproductive cycle in the same individual and most of the five stages in all samples with length greater than 16 mm (adults), suggests that the population was reproductive throughout the year (Figs. 6, 7). There were marked spawning peaks in July-August/74; September-October/74; November-December/74; May-April/75 and July-August/75. However, the annual cycle appeared to be divided in two reproductive periods: January to June and July to December (Fig. 7). There was greater reproductive activity during July-December, when most individuals apparently did not pass consecutively through five stages, but rapidly restarted the reproductive cycle in the LiS (Fig. 3F) after PS. Both males and females reorganized residual gametes and restarted a new cycle of gametogenesis. Nearly 70% of samples contained mature and spawning stages, whereas only 40% were reproductive between January and June (Fig. 7). During this period most individuals restarted a new cycle of gametogenesis in the EAS and US stages (Fig. 3A, E) after PS, increasing the frequencies of EAS and LiS (Fig. 7).

Recruitment

Monthly recruitment of spat measuring 0.1 to 4.0 mm showed minimum densities from January to June, and maximum densities from July to December (Fig. 8). During this period there were peaks in September, October and December of 1974, coinciding with the observed gonadal changes (Fig. 7).

DISCUSSION

Certain generalizations about the reproductive biology of *D. denticulatus* and the structure of its populations in the study area are possible. Sexual differentiation appears to be rapid and distinct in individuals, but differentiation in the cohort appears to be slow and asynchronous. For example, juveniles show progressive sexual differentiation between 9 and 17 mm. According to the Von Bertalanffy growth equation established for this population (Vélez et al., 1985) these sizes should be reached at 3 and 7 months respectively. Thus, sexual differentiation in a cohort can last about 4 months. Afterwards, most clams sampled showed signs of having spawned, suggesting that sexual maturation requires approximately 7 months. If the life expectancy of *D. denticulatus* is around 13-16 months (Wade, 1968 and Vélez et al., op. cit.), and adult has a total reproductive activity of only 6 months; this reproductive period is relatively short when compared with those of the oyster *Crassostrea rhizophorae* and the mussel *Perna perna*, other tropical bivalves with similar life cycles (Vélez, 1976; Vélez and Epifanio, 1981). Sex ratios indicate that recruitment and mortality rates of the two sexes are similar.

The bimodal reproductive activity with reproduction lower is January to June than in July to December, is explained by decreased gametogenesis and dominance of juvenile classes which direct their energy toward growth (Wade, 1968; Vélez et al., op cit.). In July to December the population increases gametogenesis as a result of both the tendency of individuals to activate and synchronize gonadal events, and the predominance of adults (Ibid.). During that period recruitment appears to modify, to a large extent, the population structure (Vélez et al., op cit.). Recruits resulting from this part of the annual reproductive cycle will participate in the active reproductive period of the population during the following year. Most of the individuals that spawned during the reproductive period die by the following January or February (Wade, 1968 and Vélez et al., op. cit.).

Similarity between the annual reproductive pattern in the Venezuelan population and two

Jamaican populations (Wade, 1968) suggests that the cycle is relatively independent of local environmental conditions, such as wind patterns and fluctuations in salinity. The importance of temperature fluctuations in the timing of reproduction in tropical bivalves has been pointed out by Penzias (1969) and Vélez (1977), and supported by experiments with *Perna perna* (Velez and Epifanio, 1981). Temperature variations of interstitial water in the substrate habitat of *D. denticulatus* are minimal from December to May (22 to 25°C), and maximal from July to November (27 to 29°C). The period with maximum temperatures and greatest diurnal temperature variations corresponded with the period of greatest reproductive activity and recruitment. Conversely, the period with coolest temperatures and minimum diurnal variations coincided with decreased production, spawning and recruitment.

In the Caribbean, we may distinguish two complexes of bivalves. One is characteristic of the tropics, accumulating glycogen reserves during those months when temperatures are at a minimum (22°C). Spawnin and recruitment for this group are optimal when temperatures are highest. This group includes the oyster *Crassostrea rhizophorae* (Vélez and Bonilla, 1972; Vélez, 1977), *Modiolus modiolus squamosus* (Flores, personal communication) and *D. denticulatus*. The other group, represented by the bivalves *Perna perna* and *C. virginica*, is of temperate origin and populations in the Caribbean represent the northern and southern extremes of their geographic distribution. Their energy reserves accumulate when temperatures are at a maximum and their reproduction is most intense when temperatures are lowest (Benítez, 1968; Carvajal, 1969; Lunetta, 1969; Salaya, 1977). Thus, there appear to be two species complexes of bivalves in the Caribbean having opposite temperature requirements for their reproduction. However, more experimental work is necessary to test this hypothesis,

ACKNOWLEDGEMENTS

We appreciate the assistance provided by Mr. Antonio Sotillet in the collection of the samples and Dr. Wouter Zurburg for reviewing the manuscript. This work was supported by the Research Council of the Universidad de Oriente of Venezuela.

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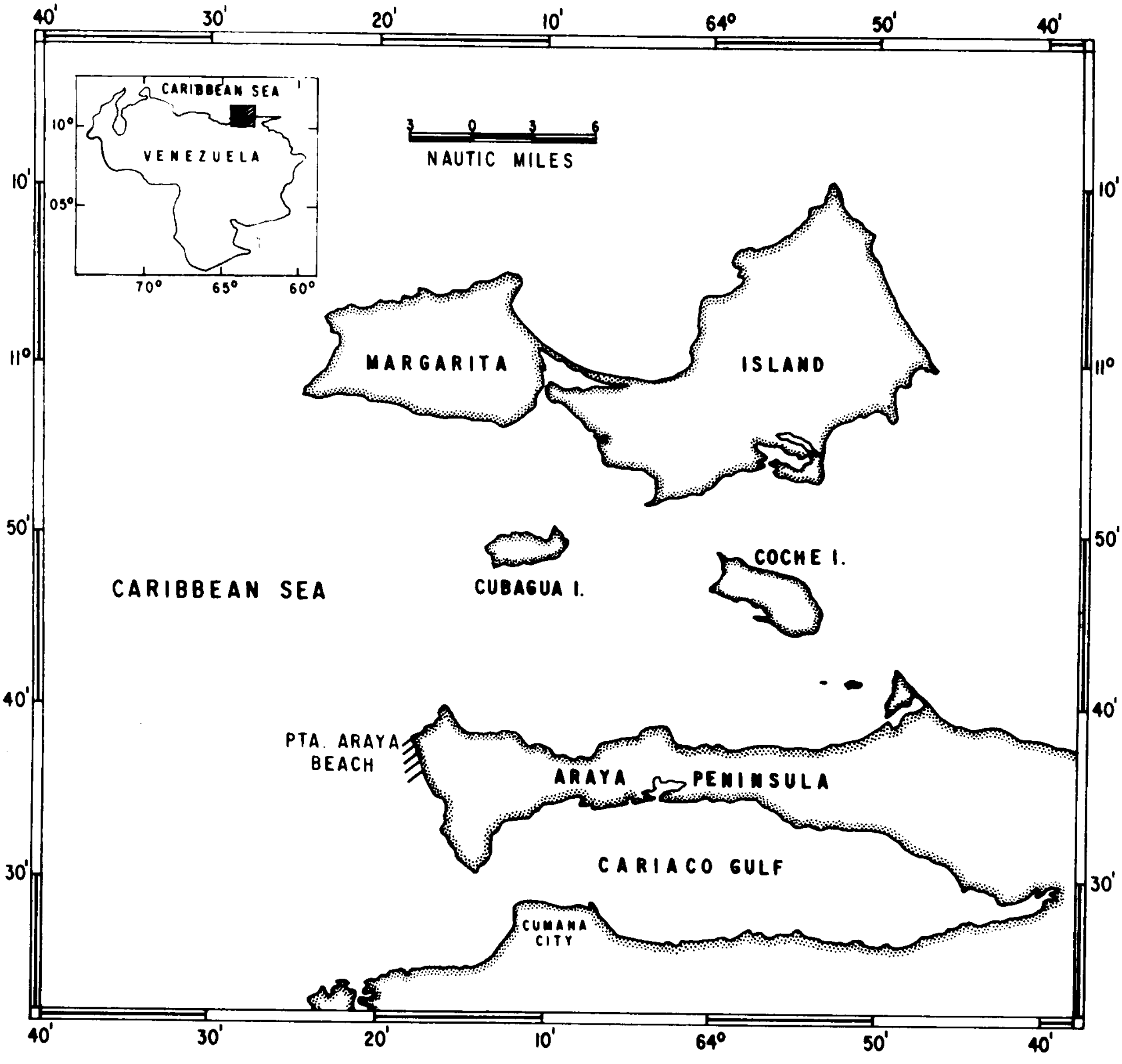

FIG. 1. – Location of Punta Araya beach in Araya Peninsula, eastern Venezuela.

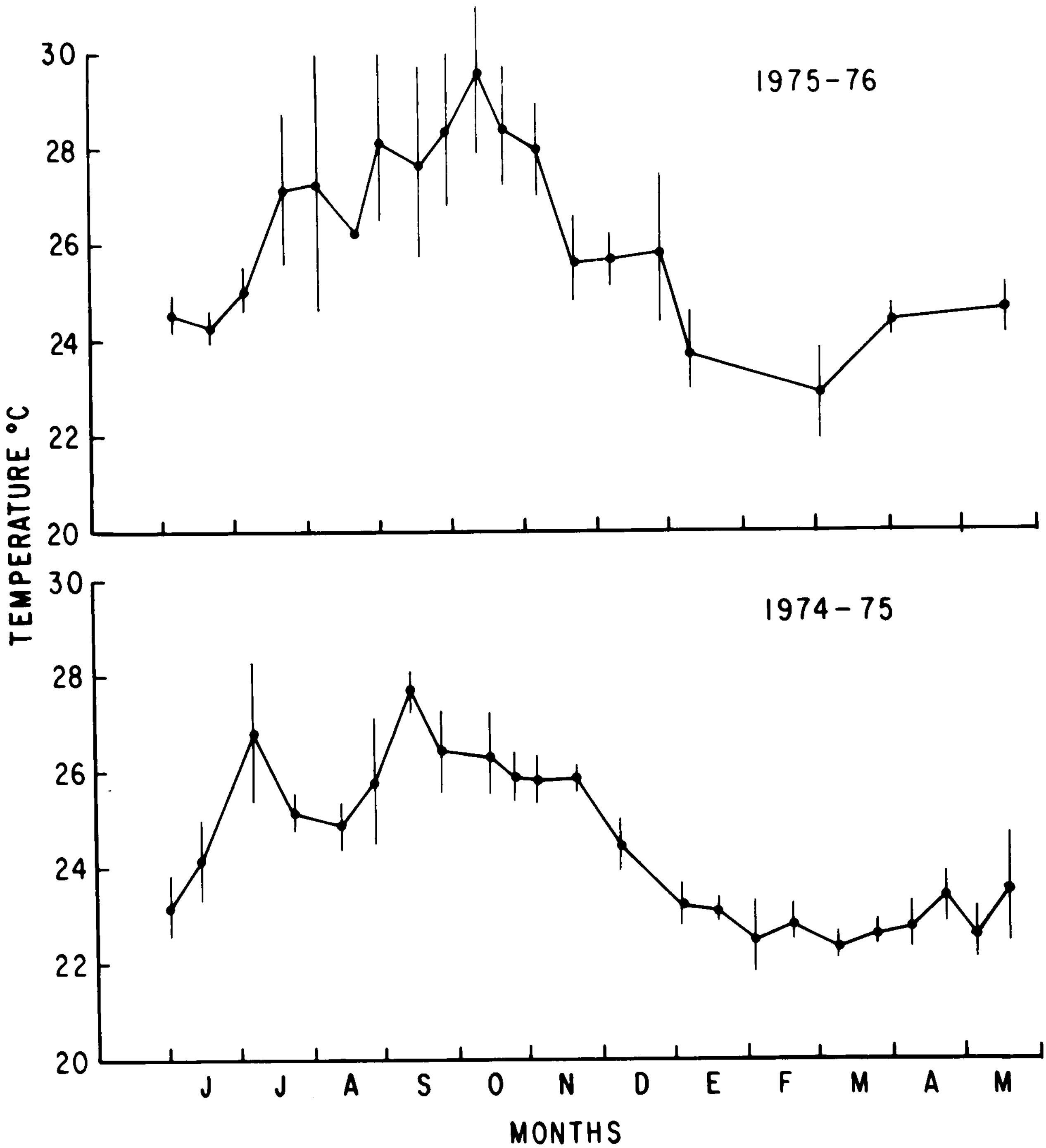

FIG. 2. – Diurnal interstitial water temperature; vertical lines are ± one standard deviation.

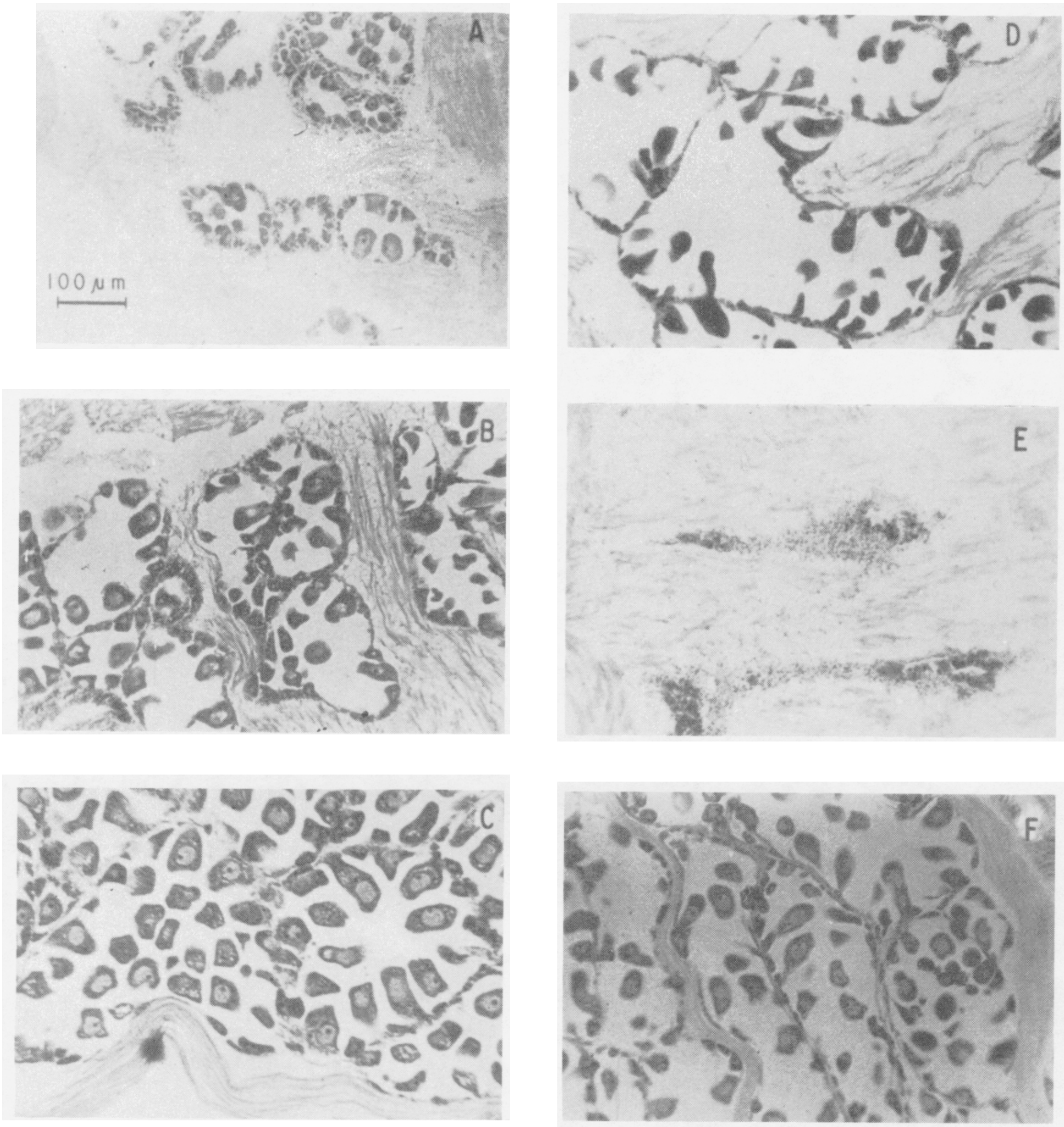

FIG. 3.– Sections of gonadal tissue from female *Done. denticulatus in* each stage of its reproductive cycle, A- early active stage (EAS); B- Late active stage (LiS); C- mature stage (MS): D- partially spawned stage (PSI; E- undifferentiated stage (US); F- late inactive stage (PSI.

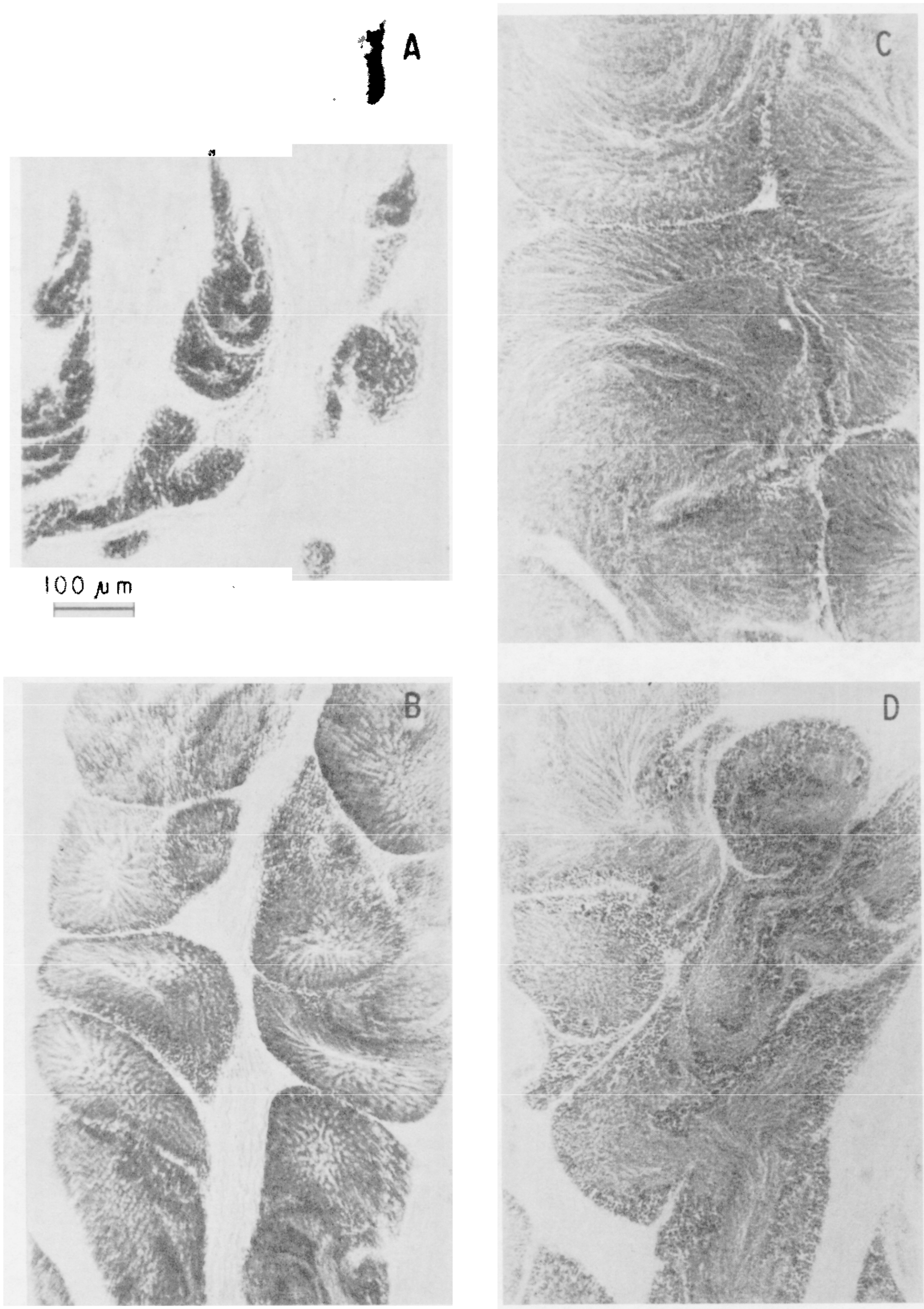

FIG. 4.– Sections of gonadal tissue from male *Donax denticulatus*. A- early active stage (EAS); B- late active stage (LiS); C- mature stage (MS): D- Partially spawned stage (PS).

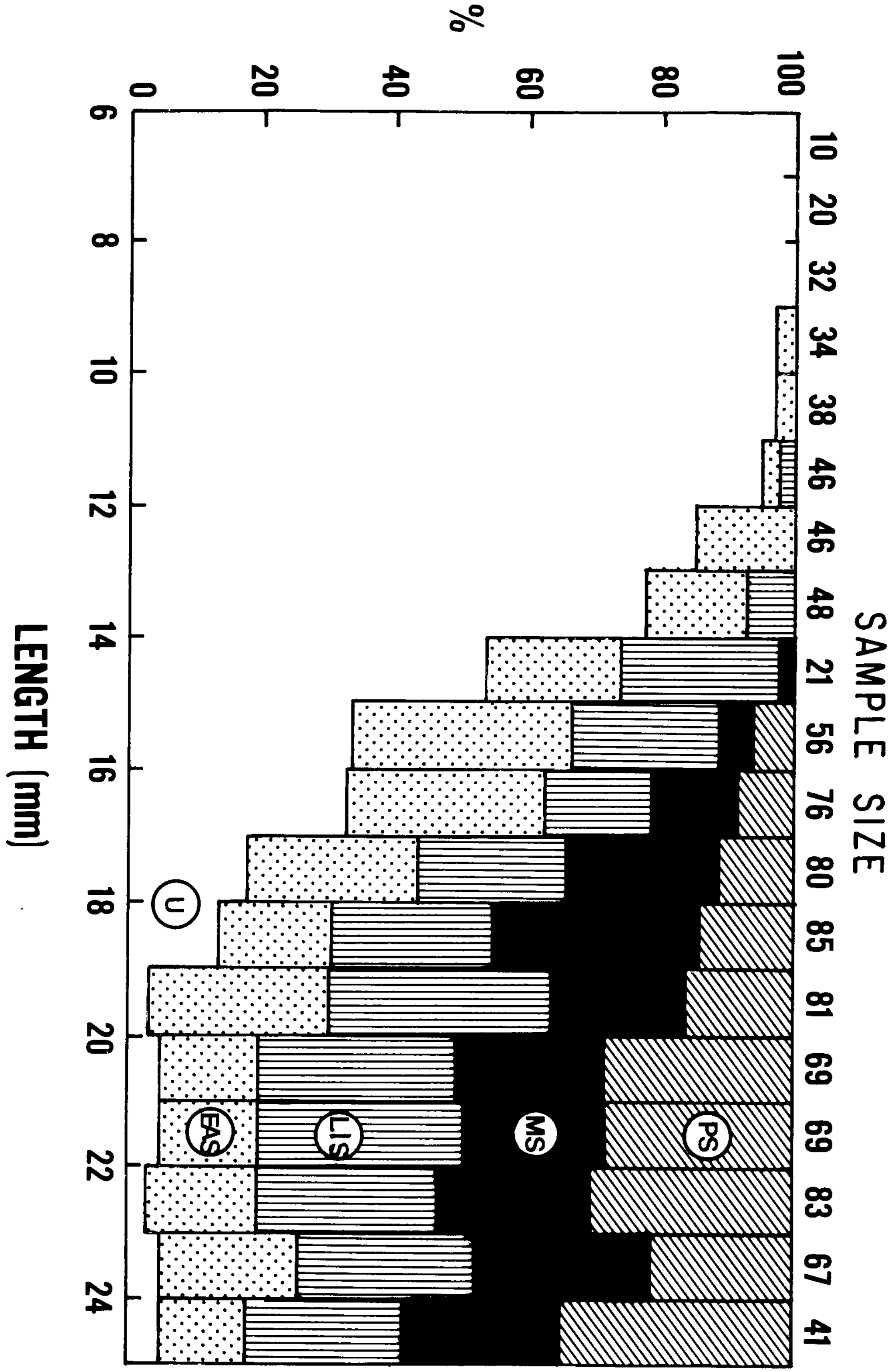

FIG 5. – Percentages of *Donax denticulatus* in each stage of the reproductive cycle for each class. The length of each shaded area represents the percentage frequency of clams in each developmental stage. The number of clams per class are shown at the top of the diagram. U- undifferentiated stage.

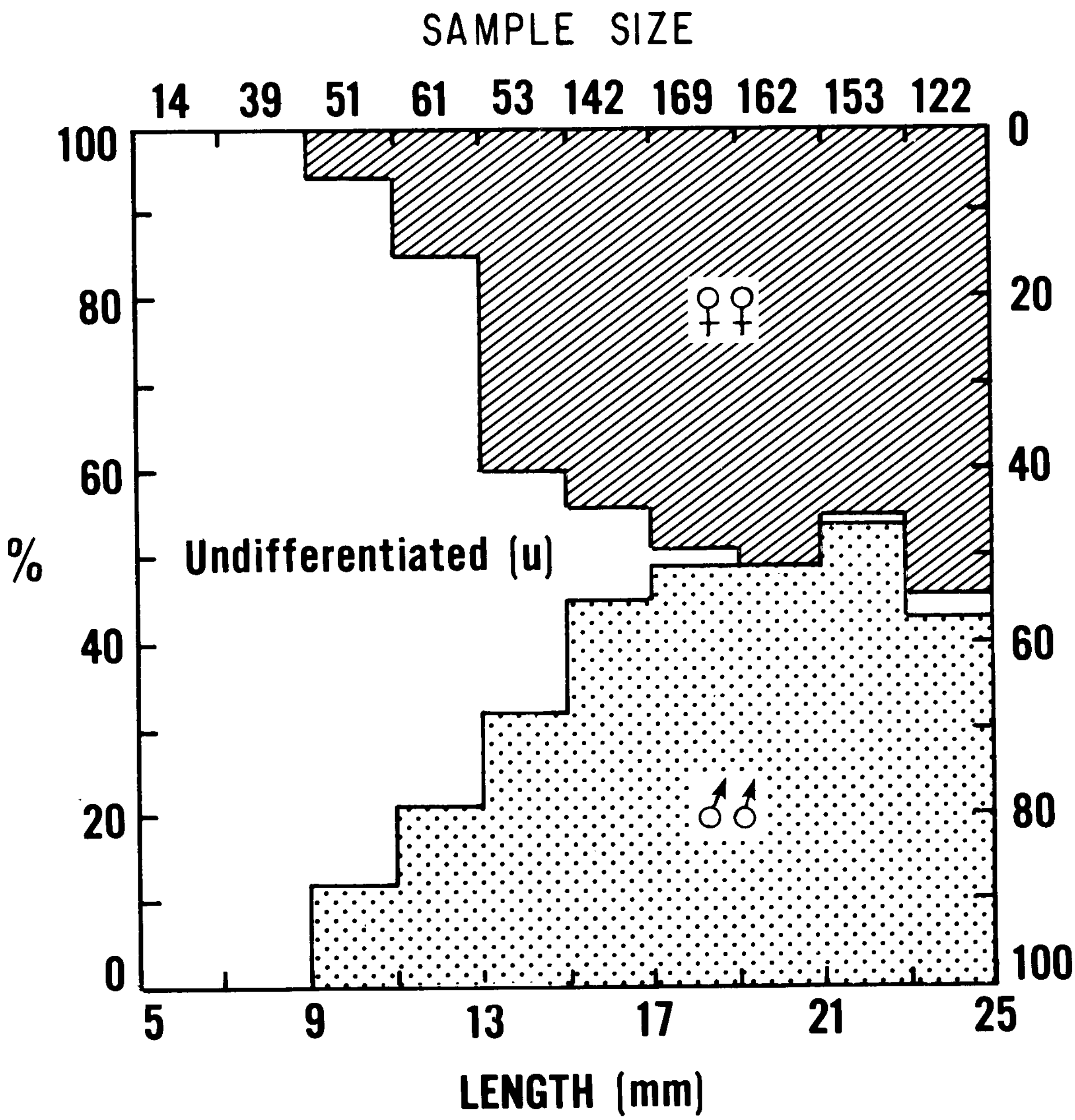

FIG 6. – Sex ratios of *Donax denticulatus* in each size class. The number of clams in each size class is shown at the top of the diagram.

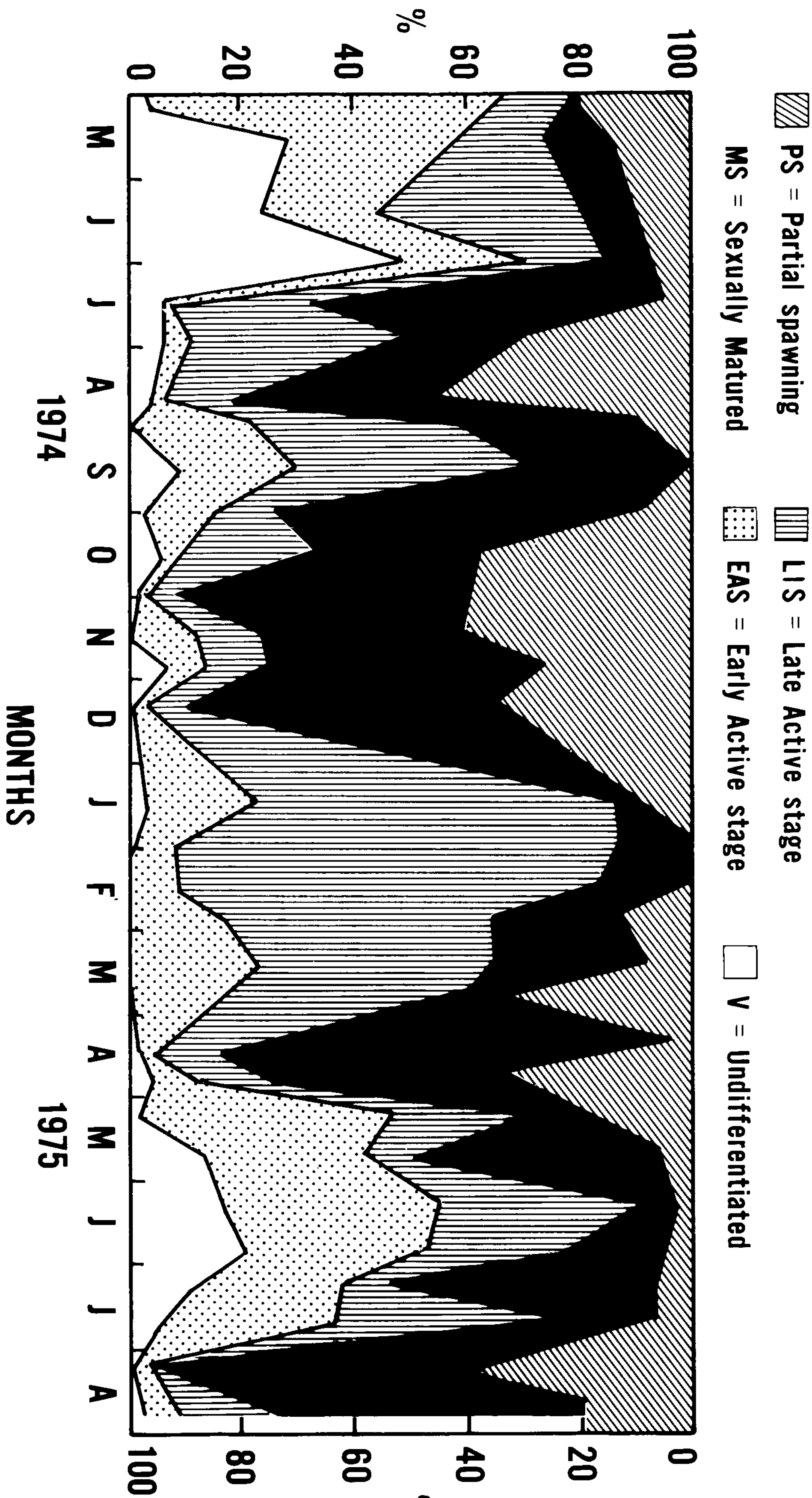

FIG. 7.—Percentages of *Donax denticulatus* in each stage of the reproductive cycle over a period of sixteen months. Sample size per month was 35 adults.

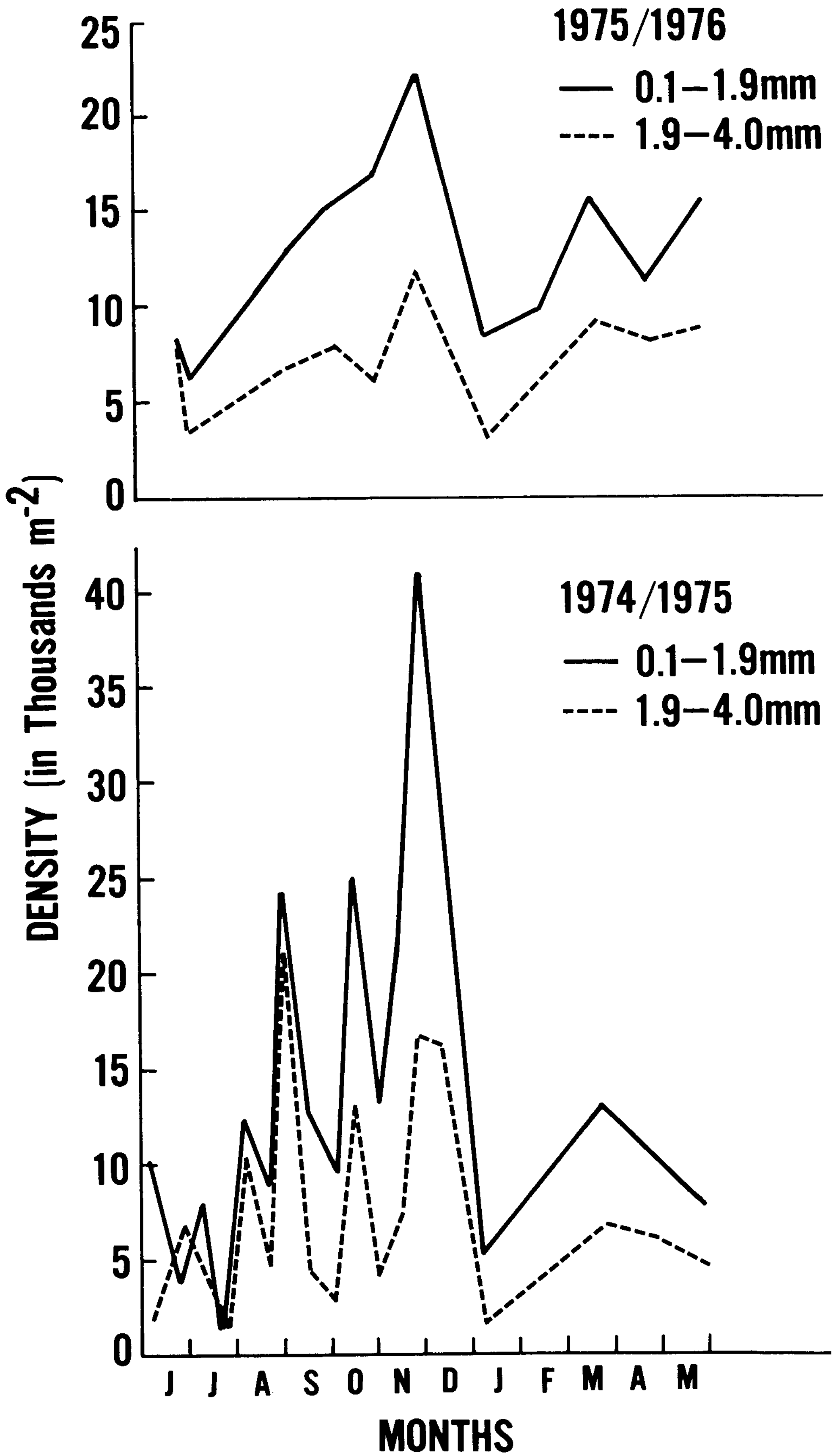

FIG. 8.—Recruitment of *Donax denticulatus* during the period of study. The values are the average number of spats for four stations.