

## THE REPRODUCTIVE CYCLE OF THE SUNRAY VENUS CLAM *Macrocallista nimbosa* (LIGHTFOOT 1786)

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### ABSTRACT

The annual reproductive cycle is described for the sunray venus clam, *Macrocallista nimbosa* (Lightfoot). Clams used in the study were collected from waters adjacent to Blacks Island in St. Joseph Bay, Florida. The reproductive activity was determined by histological examination of gonadal sections, by monitoring variation in the glycogen content of the tissues, and by variation in strip spawning potentiality.

Results from the histological study indicate that spawning during 1974 began in July for males and continued through December, with peak spawning occurring during November. Females began spawning in August and continued throughout November, reaching peak spawning activity during October and November.

The reproductive activity was reflected in the seasonal variation in glycogen content, with greatest glycogen storage occurring during the winter, averaging 15%, then reaching an annual low value of 3.8% in July, with a slight rise in August and September and a drop to 5.1% during October, returning to high winter values again in December.

Clams were strip-spawned throughout the year, but viable larvae were obtained only during October and November.

### INTRODUCTION

The clam fishery in Florida has been dominated by three venerid species, the northern quahog, *Mercenaria mercenaria* (L.); the southern quahog, *M. campechiensis* (Gmelin); and the sunray venus clam, *Macrocallista nimbosa* (Lightfoot) (Godcharles and Jaap, 1973). The fishery for the sunray venus clam was initiated in February 1967 near Port St. Joe, Florida, but at present, the fishery is inactive because insufficient numbers of clams are available. The present study was undertaken to provide basic information on the biology of this potential mariculture organism.

This paper presents the first published description of the reproductive cycle of the sunray venus clam. Earlier investigators used a variety of

research methods, either singly or in combination to determine the reproductive activity in bivalves. Three methods were used in this study:

(1) histological examination of gonadal sections; (2) variation in glycogen content of tissue; and (3) variation in strip-spawning potentiality. This study compares and contrasts the suitability of these methods in ascertaining the seasonal reproductive state of *M. nimbosa*.

### MATERIALS AND METHODS

The study began in January and was completed in December 1974. The clams were collected in waters 300 meters north of Blacks Island in St. Joseph Bay, Florida in depths ranging from 1.0 to 1.5 meters at mean low tide. Collections for the histological study were made once a month except

for the months of July and August when two collections were made per month. The sample size ranged from 10 to 23 clams per month.

Within 24 hours of collection, a 10-mm cube of gonadal tissue was removed from the mid-lateral portion of the visceral mass of each clam and preserved in Bouin's fixative. After 7 days in Bouin's fixative, the tissues were transferred to 50% ethanol and held for further histological processing. Fixed gonadal tissues were dehydrated in alcohol, cleared in xylene, and embedded in paraffin. The embedded tissue was then sectioned at 8 microns, mounted on a slide, stained in Erlich's hematoxylin, and counterstained with alcoholic eosin.

Each slide was examined microscopically and a random sample of 20 alveoli per slide was assigned to a category of gonad condition. The histological study of the reproductive cycle is based upon examination of 4,000 alveoli. The cyclic reproductive process is divided into 5 phases of gonad condition which apply to both sexes: early active, late active, ripe, partially spawned, and spent. These 5 phases and their distinguishing characteristics are used by various investigators, e.g., Ropes and Stickney (1965) for *Mya arenaria*, Ropes (1968) for *Spisula solidissima*, Cain (1972) for *Rangia cuneata*, and Holland and Chew (1974) for *Venerupis japonica*. There is no sharp distinction between phases and the categories are convenient rather than natural (Ropes, 1968).

The method used in this study of examining 20 alveoli per slide or individual is different from the methods of other investigators who assign each clam or slide to one of the 5 phases. A new procedure was used for the sunray venus clam because one clam may contain alveoli in several different phases, and to assign the individual clam to just one phase would have masked the other phases present. It is also believed that maturation of alveoli from one phase to another can occur within a few days time, and that individual clams do not empty all of their gametes at one spawning, so it is informative to express the proportion of alveoli in the various phases.

The five phases of female gonad condition and their distinguishing characteristics are described:

(1) *Early Active Phase*. Ovogonia occur at the periphery and within the alveolar walls. Follicle cells frequently occur within the alveolus.

(2) *Late Active Phase*. Alveoli in the late active phase contain a large number of elongated stalked ovocytes, whose free ends protrude into the alveolar lumen and whose bases attach to the alveolar wall. The large, stained ovocyte nucleus is a conspicuous characteristic of this phase and basophilic nucleoli are present. The basement membrane is thin.

(3) *Ripe Phase*. The alveolus is termed ripe when the number of ova free within the lumina exceeds the number of attached ovocytes (Cain, 1972). The attached ovocytes resemble those in the late active phase, except that in the ripe phase, amphinucleoli are present. Ripe alveoli are filled with ova and ovocytes and appear crowded together.

(4) *Partially Spawned Phase*. A few ovocytes are still attached to the thickened alveolar walls; a few residual ripe ova may remain in the alveolar lumen.

(5) *Spent Phase*. Alveoli are usually empty of ripe ovocytes; those that are present are usually undergoing cytolysis. The alveolar walls are thickened and oogonia are often present. The spaces between alveoli are filled with mesenchyme.

The stages of male gonad condition are described:

(1). *Early Active Phase*. Alveolar walls are thickened and contain darkly stained spermatogonia. Primary spermatocytes are found at the alveolar periphery and begin to proliferate toward the lumen. Follicle cells may fill the alveoli. Alveoli contain nutritive phagocytes (Loosanoff, 1937).

(2). *Late Active Phase*. This phase is characterized by the proliferation and maturation of spermatocytes. The spermatocytes are uniformly shaped cells which at the initiation of maturation are found near the alveolar periphery, but as development progresses, migrate toward the alveoli centers. Later in the active phase the spermatocytes elongate and are arranged in radially aligned columns. A central lumen within the alveolus is formed. A small number of spermatozoa may be within the lumen.

(3). *Ripe Phase*. With the development of tails the spermatids are transformed into spermatozoa. Spermatozoa are arranged in radial columns in the alveoli with their tails oriented toward the lumen.

Later in this phase, masses of free spermatozoa fill the alveolar lumen.

(4). *Partially Spawned Phase*. Spermatozoa are present within alveolar centers, but are less numerous than in the ripe phase. A thin band of spermatogonia and primary spermatocytes may be found along the basal membrane before the alveolus is empty of spermatozoa.

(5). *Spent Phase*. Alveoli in the spent phase contain few or no spermatozoa and the lumina are open.

The glycogen content of clam tissue was determined by the colorimetric phenol method (Westenhouse, 1968). Clams used for glycogen analysis were processed within 24 hours of collection. Five clams ranging from 120 to 130 mm in length were analyzed each month for their glycogen content. The glycogen content for each month is a mean of three replicate determinations.

The strip-spawning potential was checked biweekly during the first 5 months of 1974, then checked at weekly intervals for the remainder of the year. The gametes were procured by methods described by Loosanoff and Davis (1963). During each test the gametes from 5 different females and 5 different males were pooled, and half of the gamete solution received 15 ml 0.1N  $\text{NH}_4\text{OH}$

treatment to dissolve the ovum germinal vesicle. Three hours after the addition of sperm to the egg suspension, the eggs were microscopically examined for evidence of cleavage. If cleavage did occur, the cultures were continued and examined 48 hours after fertilization.

## RESULTS AND DISCUSSION

### *Histological Study*

Histological examination of the 1974 female reproductive cycle revealed a single annual spawning period which began in August and continued through November with peak spawning activity occurring during the fall months of October and November (Fig. 1).

All five gonadal phases were present throughout the winter and spring months, January to May, with little change occurring in the proportion of the phases throughout this period.

Females contained at least some ripe alveoli nine months of the year, January through September, with ripeness peaking during the summer months of June and July, 52 and 54% respectively, and then declining to very few or no ripe alveoli during October through December.

Females contained alveoli in partially spawned and spent phases throughout the year. There was

### FEMALE

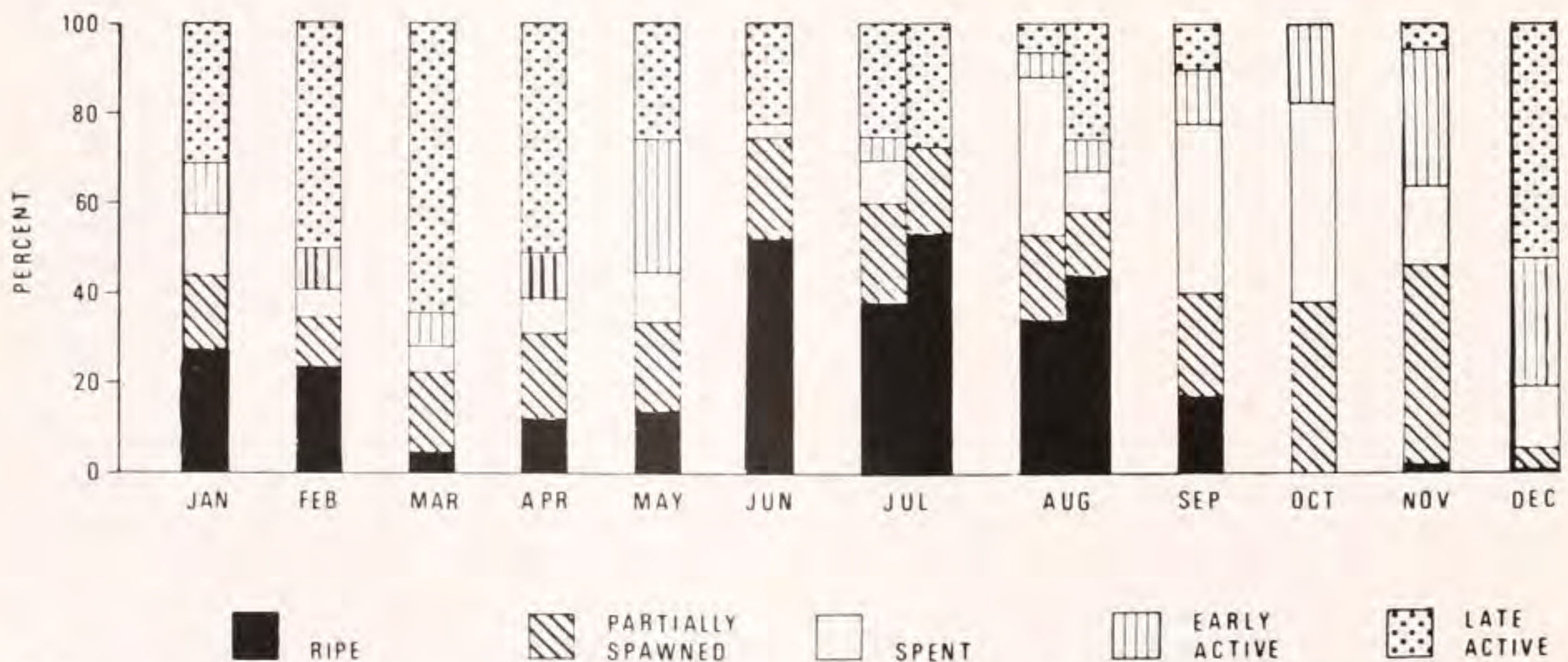


FIG. 1. The female reproductive cycle of the sunray venus clam, *Macrocallista nimbosa* during 1974 from St. Joseph Bay, Florida. The length of each shaded area represents the percent frequency of alveoli in each reproductive phase.

little change in the proportion of these two phases during the first seven months of the year. During this time alveoli in partially spawned and spent phases represented 25% of the total alveoli.

In August, there was a sharp increase to 54% in the proportion of alveoli in the partially spawned and spent phases. This proportion increased further in September to 61% and reached an annual maximum of 82% in October, remaining high at 62% in November. There was a sharp decline in spawning activities to the annual minimum of 18% alveoli in the partially spawned and spent phases by the first week of December.

Active phases, which include early active and late active, were present throughout the year. During the first five months of the year alveoli in the active phase represented a high percentage, averaging 58% of the total. During the summer and early fall this proportion was reduced, reaching a yearly minimum in August. The proportion of alveoli in active phases increased in the fall, reaching an annual maximum of 81% in early December.

Males likewise exhibited a single annual spawning period (Fig. 2). Some males contained ripe alveoli throughout the year, averaging 40% for the first five months, then sharply increasing to the annual maximum of 98% in June. Beginning in

July and August the percentage of ripe alveoli steadily declined throughout the fall and reached the annual minimum of 3% in December.

There was little change in the proportion of alveoli in the partially spawned phase throughout the first five months of the year. Alveoli in the partially spawned phase were absent during June, but then reappeared and were present throughout the remaining months of the year with alveoli in the partially spawned and spent phases reaching maximum annual values of 68 and 86% during November and December, respectively.

Alveoli in the spent phase first appeared in May and June and were present for the remaining months of the year.

Except for the month of June, active phases were present throughout the annual cycle. There was little change in the proportion of active phases during the winter and spring months, as well as during September and October. The percentage of alveoli in active phases decreased as fall progressed and reached a low in December.

This histological study indicates that the sunray venus clam is a fall spawner, with females beginning spawning in August and continuing through November, with greatest spawning activity occurring during October and November. Males begin spawning activities earlier, starting in July

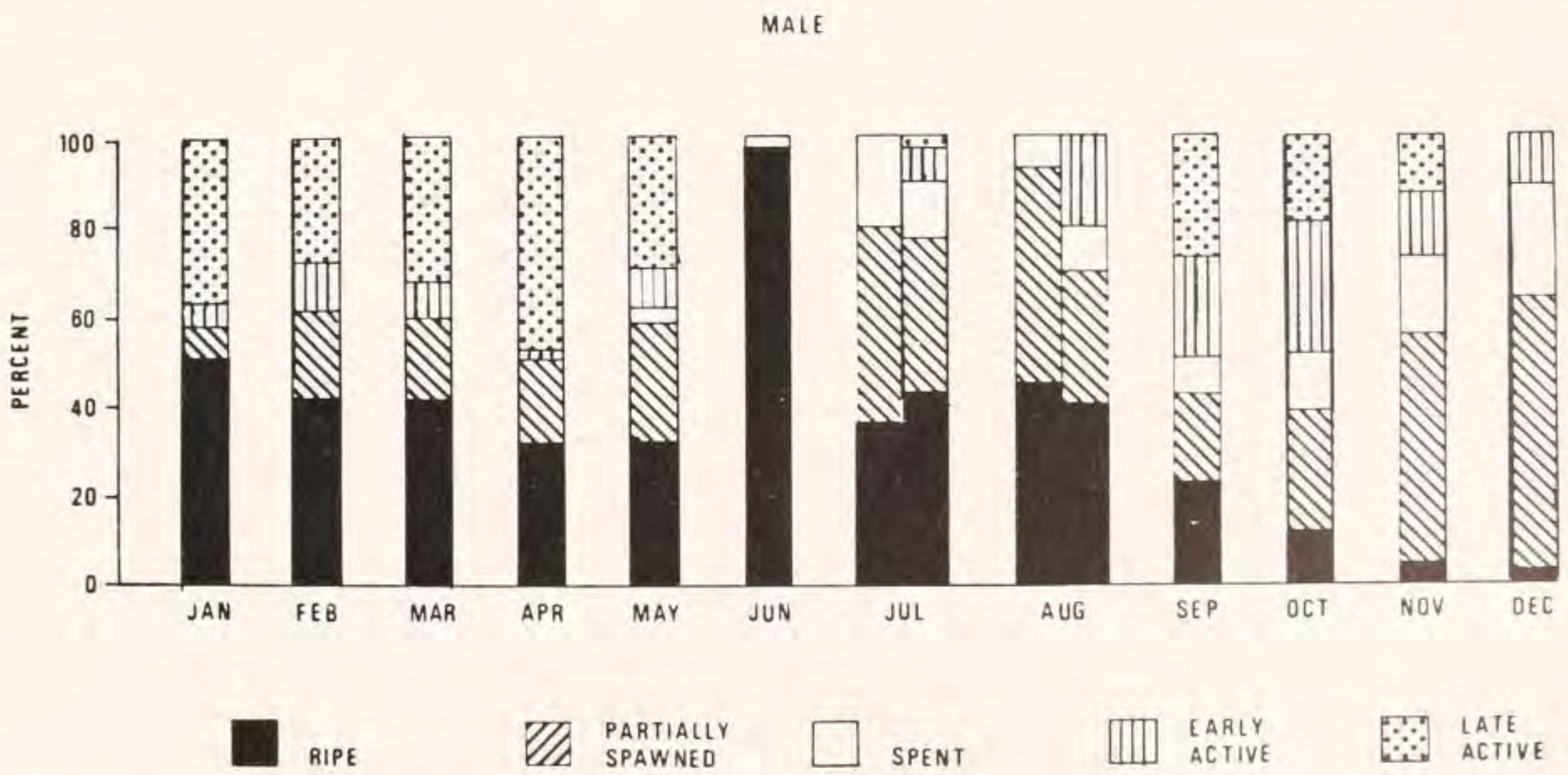


FIG. 2. The male reproductive cycle of the sunray venus clam during 1974.

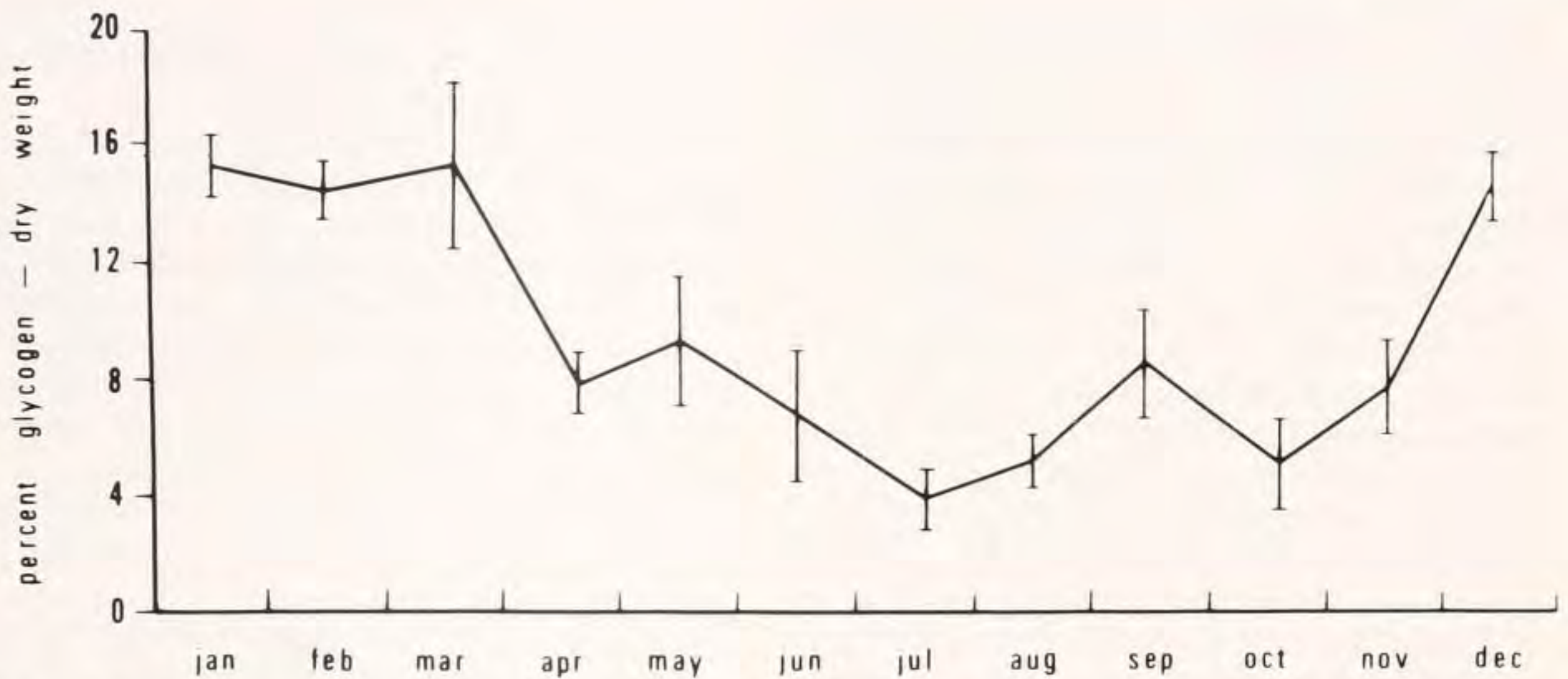


FIG. 3. The seasonal variation in glycogen content (on a dry weight basis) for the sunray venus clam. The vertical bars indicate 95% confidence intervals.

and continuing through early December, with greatest activity occurring from October to early December.

#### Glycogen Analysis

The greatest storage of glycogen in the tissues of the sunray clam occurred during the winter months of January through March (Fig. 3). The annual maximum value of 15.2% occurred in both

January and March. A sharp decline in glycogen content during April continued throughout the months of May and June, reaching low values of 3.8 and 5.2% during the summer months of July and August respectively. There was a transient rise in glycogen content during September. Glycogen levels returned in October to a value of 5.1%, reminiscent of July and August. Glycogen

TABLE 1. The annual strip spawning potential of *M. nimbosa*. The numbers in parentheses indicate the number of trials in which ova development was successful.

Month	Number of Trials	Ammonium Hydroxide Treatment		No Treatment	
		Development To		Development To	
		Early Gastrula Or Less	Straight-hinge Stage	Early Gastrula Or Less	Straight-hinge Stage
Jan.	2	—	—	—	—
Feb.	2	—	—	—	—
Mar.	2	+(1)	—	—	—
Apr.	2	—	—	—	—
May	2	+(1)	—	—	—
Jun.	4	+(1)	—	—	—
Jul.	4	—	—	—	—
Aug.	4	—	—	—	—
Sep.	4	+(2)	—	—	—
Oct.	4	+(3)	+(3)	+(3)	+(3)
Nov.	4	+(4)	+(4)	+(4)	+(4)
Dec.	4	+(1)	—	—	—

content rose slightly in November and then sharply in December to 14.4%, a value comparable with the high winter values of January through March, thus demonstrating a definite annual glycogen cycle for *M. nimbosa* during 1974.

The seasonal changes in the glycogen content of *M. nimbosa* showed a definite cycle related to the gonadal development and spawning activities.

During the winter months, January through March, the glycogen values were at the annual high and little change occurred in the proportion of the five gonadal phases. In June, the histological study revealed a rapid proliferation of gametes and a corresponding low value of glycogen which decreased to an annual minimum in July at a time when spawning began in males. The correlation between low glycogen values and spawning continued throughout November. Glycogen content showed a small rise in September which was reflected in the histological study in that a corresponding decrease in alveoli in the spawned phases occurred. By December, spawning had ceased and an increase in glycogen approaching the winter values was observed in December.

#### *Strip-Spawning Potential*

The annual strip-spawning potential is represented in Table 1. Development of gametes to and beyond the straight-hinge stage occurred only in those experiments conducted during the last three weeks of October and all of November. During this period normal development occurred in both those egg suspensions treated and not treated with ammonium hydroxide. In strip-spawning experiments performed at other times of the year, eggs showed limited development only in those gamete suspensions receiving ammonium hydroxide treatment. In experiments conducted on March 14, May 3, and June 16, a few eggs developed only as far as the 8-cell stage. In experiments conducted September 13, 21, and December 3, several eggs developed to the early gastrula stage.

It is likely that the effect of the ammonium hydroxide is to break down the egg's germinal vesicle so that fertilization may occur (Loosanoff and Davis, 1963). The incomplete development of those eggs may indicate that the eggs were not mature even though sperm addition caused initiation of cleavage.

The results of this study indicate that strip-spawning is successful only during certain times of the year. Comparison of the strip-spawning and the histological study results indicate that strip-spawning is not successful throughout the entire period that an animal appears to be in a histologically-ripe or partially-spawned phase, but is confined to a narrower period than one would infer from a histological or glycogen study. The results of the study of strip-spawning potential provides further evidence that *M. nimbosa* in northwest Florida are fall spawners.

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