

Reproductive Cycle of the Coot Clam, *Mulinia lateralis* (SAY), in Long Island Sound^{1, 2}

BY

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(Plates 37, 38; 1 Text figure)

INTRODUCTION

THE COOT CLAM, *Mulinia lateralis* (SAY, 1822), a member of the family Mactridae, has received very little attention in spite of its abundance in favorable environments. This ecologically significant clam is a food of many bottom-dwelling and bottom-feeding animals, including black drum, *Pogonias cromis* (BREUER, 1957); scup, *Stenotomus chrysops*, other fishes (VERRILL, 1873); starfish, *Asterias forbesi* (DESOR, 1848), oyster drills, *Eupleura caudata* and *Urosalpinx cinerea* (SAY, 1822) (C. L. MACKENZIE, Jr., personal communication); and the greater scaup duck, *Aythya marila*, and lesser scaup duck, *Aythya affinis* (CRONAN, 1957).

Periodic histological examination of gonad tissues during several successive years has been valuable for determining the periodicity of gametogenesis in many marine invertebrates (GIESE, 1959). The reproductive cycle of many species of pelecypods has been described, but until ROPES (1968a) discussed the reproductive cycle of the surf clam, *Spisula solidissima*, gametogenesis had not been described for any of the Mactridae.

The spawning season for *Mulinia lateralis*, as indicated by the presence of larvae in plankton samples, has been reported to be from mid-July to early September at Prince Edward Island, Canada (SULLIVAN, 1948). LOOSANOFF, DAVIS & CHANLEY (1966) reported these larvae to be extremely numerous in plankton samples from Long Island Sound during late summer, but they did not attempt to define the spawning season. SHAW (1965) determined

from collections with Thorson bottles that *M. lateralis* spawns and sets in the Tred Avon River, Maryland, from May to November; peak setting is in September (HANKS, 1968).

Knowledge of the reproductive cycle of this clam is essential to an understanding of larval production and, ultimately, to the abundance of this ecologically important bivalve. The duration of the spawning season of *Mulinia lateralis* and the time of appearance of the larvae were determined by following gametogenic development in histological sections throughout the year and by making plankton studies during the spawning season.

MATERIALS AND METHODS

Mulinia lateralis were collected from several areas in the Bridgeport-Milford-New Haven, Connecticut, area of Long Island Sound. They were kept in boxes of sand placed on an underwater dock in Milford Harbor or in outdoor running water tanks at the laboratory and maintained as specimens to be sacrificed for histological study.

Ten clams were collected from this supply either weekly or in alternate weeks from August 2, 1965 to August 28, 1967, and placed in Lillie's decalcifier fixative (HUMASON, 1962) for 24 to 48 hours. The gonad was dissected out, dehydrated in alcohol, cleared in xylene, and embedded in paraffin by standard techniques. Gonad tissues were sectioned at 7 μ with a rotary microtome, stained with Delafield's hematoxylin, and counterstained with eosin. The sections were examined under an AO Spencer light microscope at $\times 100$ and $\times 430$ magnification; the gonad tissue was assigned to one of the stages of development described by ROPES & STICKNEY (1965), who categorized the seasonal cycle of gametogenesis in the soft-shell clam, *Mya arenaria* LINNAEUS, 1758, as follows: inactive, ac-

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tive, ripe, partially spawned, and spent. Photomicrographs of the various stages of gametogenesis were taken by use of a standard Zeiss light microscope and camera at $\times 200$ and $\times 500$ magnification.

To provide corroborative evidence on the beginning and duration of the spawning season of *Mulinia lateralis*, plankton samples were collected twice weekly at 5 stations in the Bridgeport-Milford-New Haven area from June 17 to October 3 and on October 8 and 24, 1968. By examining the samples I was able to determine when the larvae first appeared and to follow variations in their abundance. Samples were collected with the research vessel, *Shang Wheeler*, by pumping a 200-gallon water sample through a no. 10 bolting-silk net to screen off the larger, more easily identifiable larvae, and then through a no. 20 net to collect smaller larvae. A 12 \times 2-inch iron suction head was lowered to mid-depth with a 1.5-inch rubber hose to collect samples. The opening of the suction head was covered with fine-mesh copper screening to prevent entrance of extraneous debris. Two metal drums connected in tandem provided a system whereby water flowing through the first net was trapped in the first drum and passed through the second net through an overflow pipe. The samples were washed into 150-ml jars containing 1 ml of formalin as a preservative and later examined microscopically for the presence of *M. lateralis* larvae.

HISTOLOGICAL STUDY OF GAMETOGENESIS

The gonad in ripe *Mulinia lateralis* is a large and clearly defined organ and easily distinguishes ripe individuals from those with undeveloped gonads or those that have discharged all or most of their gametes. The gonad of ripe animals is an almost uniform, continuous mass of tissue surrounding the digestive tract and the digestive diverticula. During the spawning period the thickness of the gonad decreases as a result of the discharge of gametes. The gonad of spent animals is scarcely discernible by visual examination, but can be observed readily in histological sections.

The data from two years of monthly sampling for histological study were combined since progress of gametogenesis was essentially the same both years (Text figure

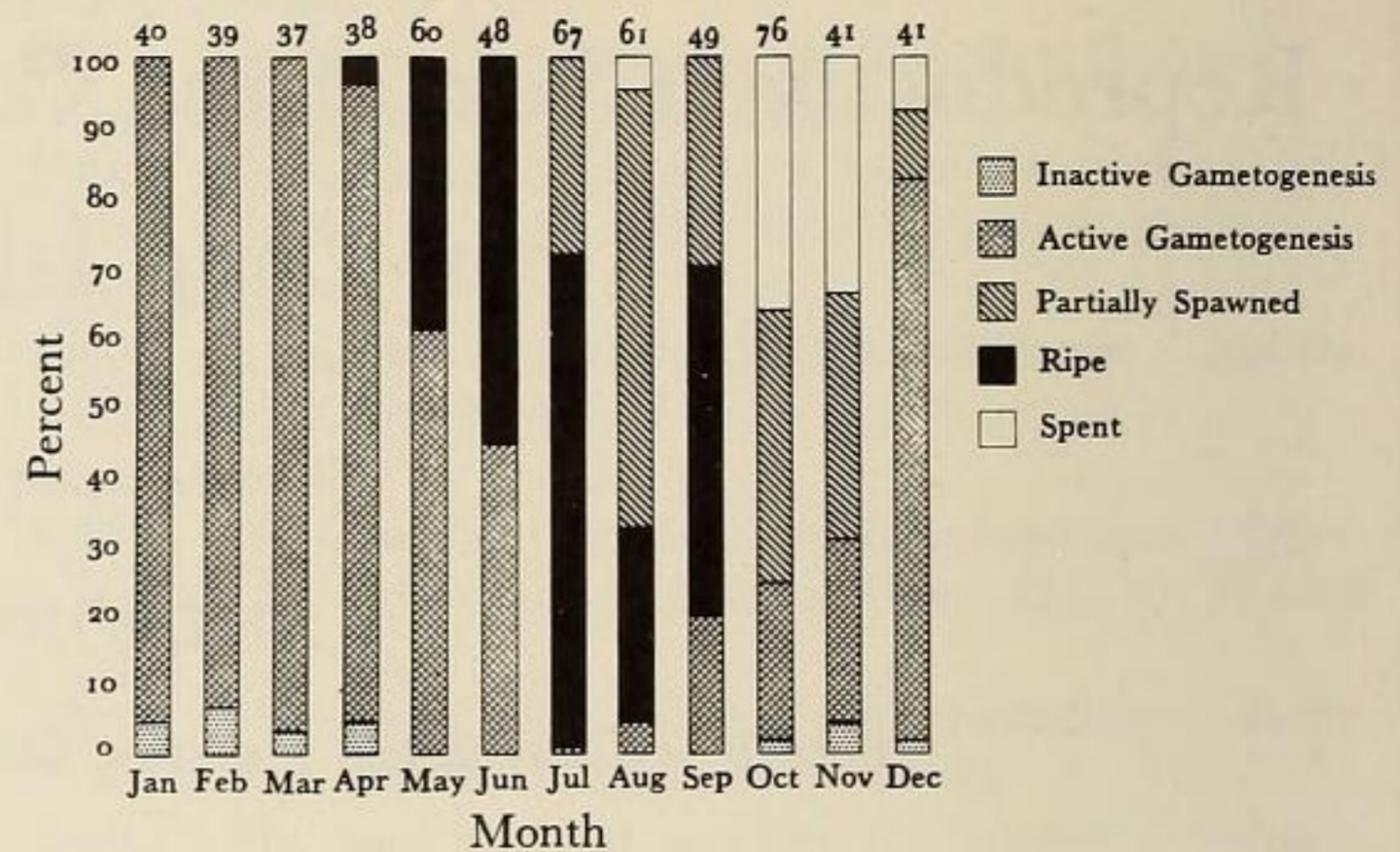


Figure 1

Gonad condition of *Mulinia lateralis* in Long Island Sound from August 1965 to August 1967. The length of each shaded area represents the percentage frequency of clams in each category. The numbers above each bar represent the total number of clams sampled that month. Data from the two years of observation are combined.

1). Histological examination of these samples indicates that spawning of *Mulinia lateralis* is completed by the end of September or early in October. No ripe unspawned individuals were found in October; 39.5% were partially spawned and 36.8% were completely spent. The gonads of spent clams examined microscopically during this period showed large, distended follicles containing few or no spermatozoa or ova within the lumina (Plate 37, Figure 5; Plate 38, Figure 11), whereas the follicles of partially spawned animals contained small numbers of ripe gametes (Plate 37, Figures 3, 4; Plate 38, Figure 10).

Sections of gonads fixed in late July and August revealed that several animals were beginning to undergo active gametogenesis. Whether this was preliminary to a second annual spawning was not determined. The gonad follicles at this time contained many spermatogenic and oogenic cells in various stages of development (Plate 37, Figure 1; Plate 38, Figure 7). The percentage of clams showing gametogenesis increased through November even though the water temperature decreased to 6° C, and by December about 80% of the animals examined were undergoing gametogenesis (Text figure 1). This activity continued at an increased level through January, February,

Explanation of Plate 37

Section of Gonad Tissue of Male *Mulinia lateralis*

Figure 1: Active phase of spermatogenesis. ($\times 500$)

Figure 2: Fully mature. ($\times 500$)

Figure 3: Partially spawned. ($\times 200$)

Figure 4: Partially spawned. ($\times 500$)

Figure 5: Spent, with few sperm retained. ($\times 200$)

Figure 6: Inactive phase. ($\times 500$)

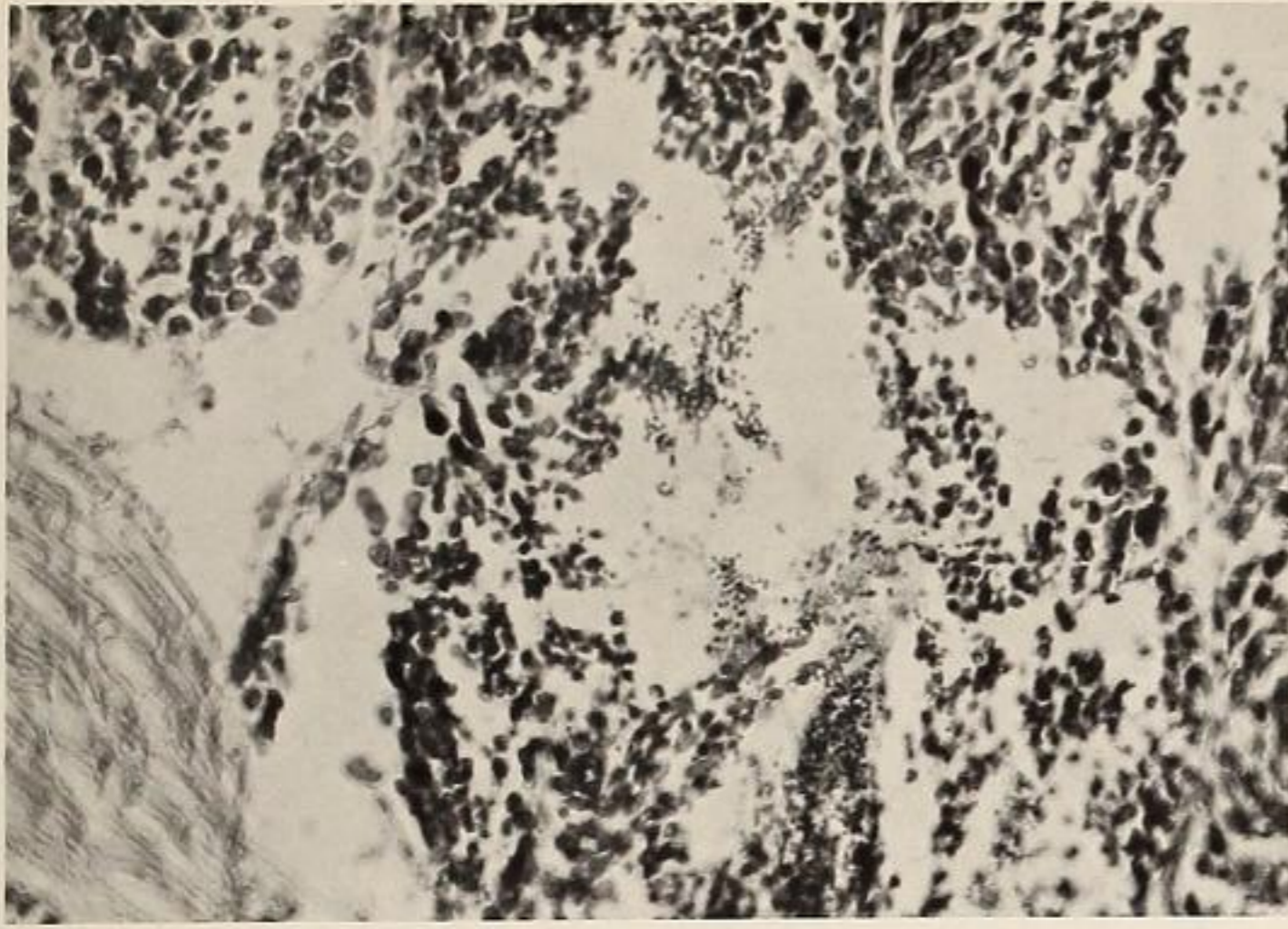


Figure 1

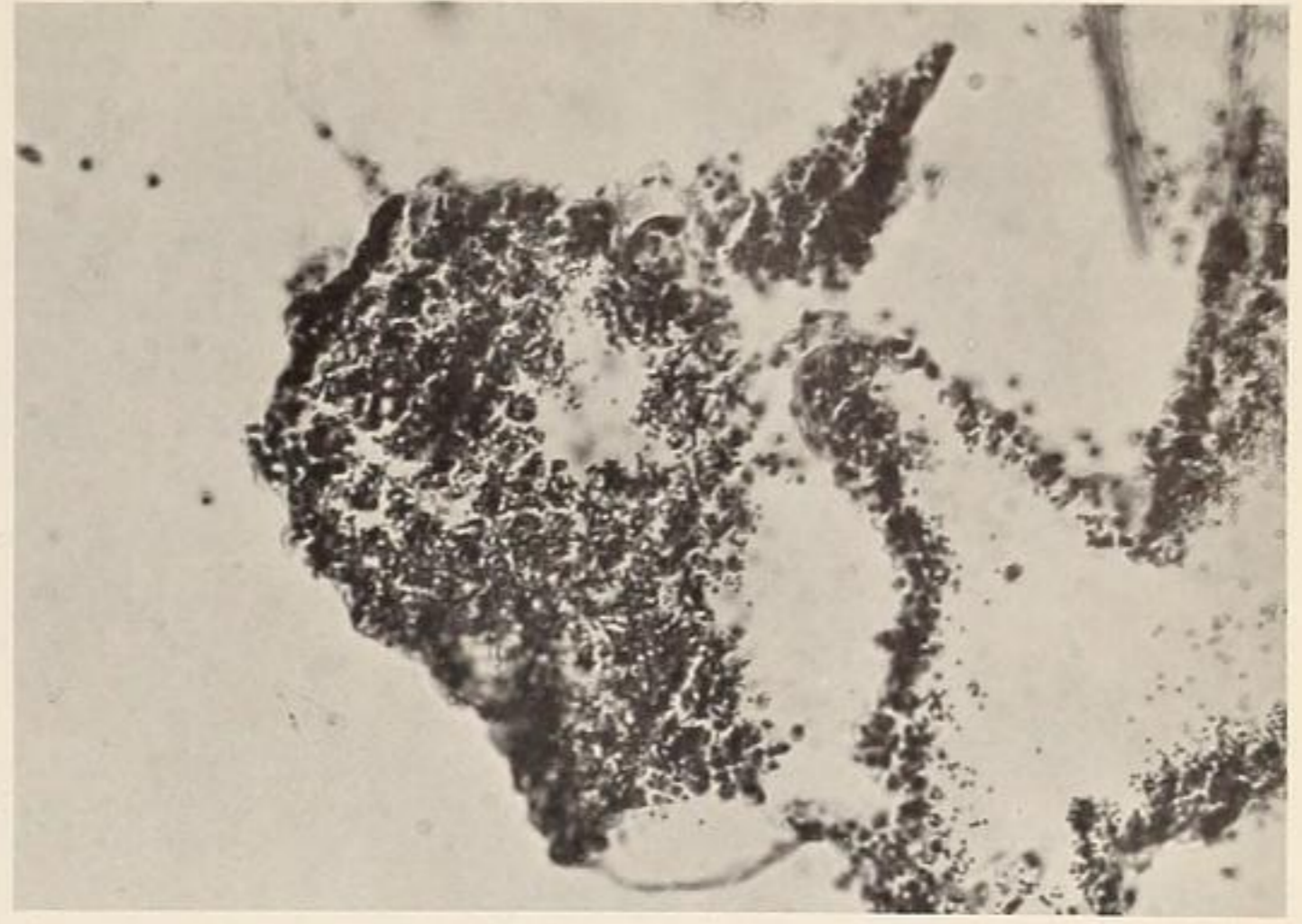


Figure 4



Figure 2

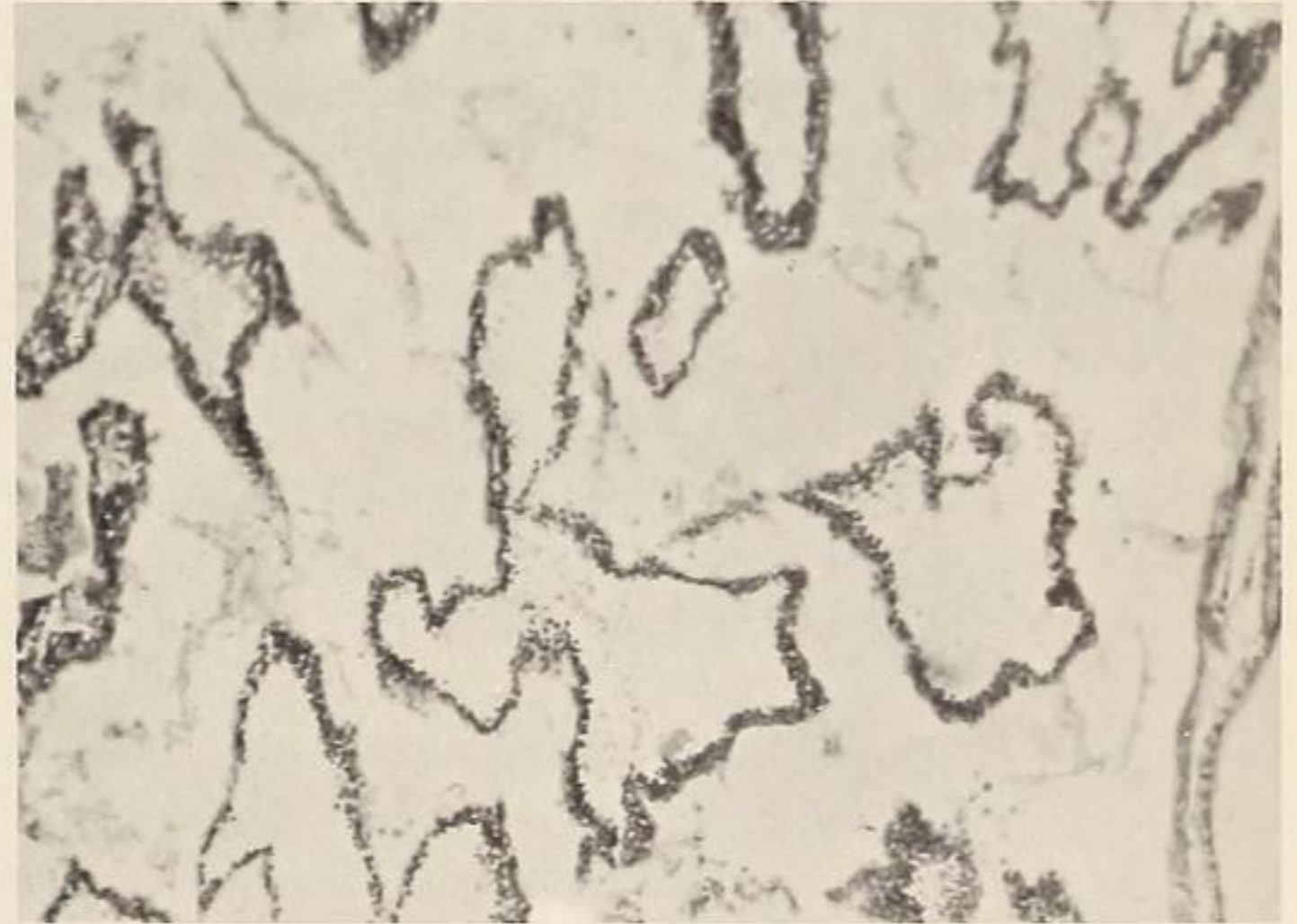


Figure 5

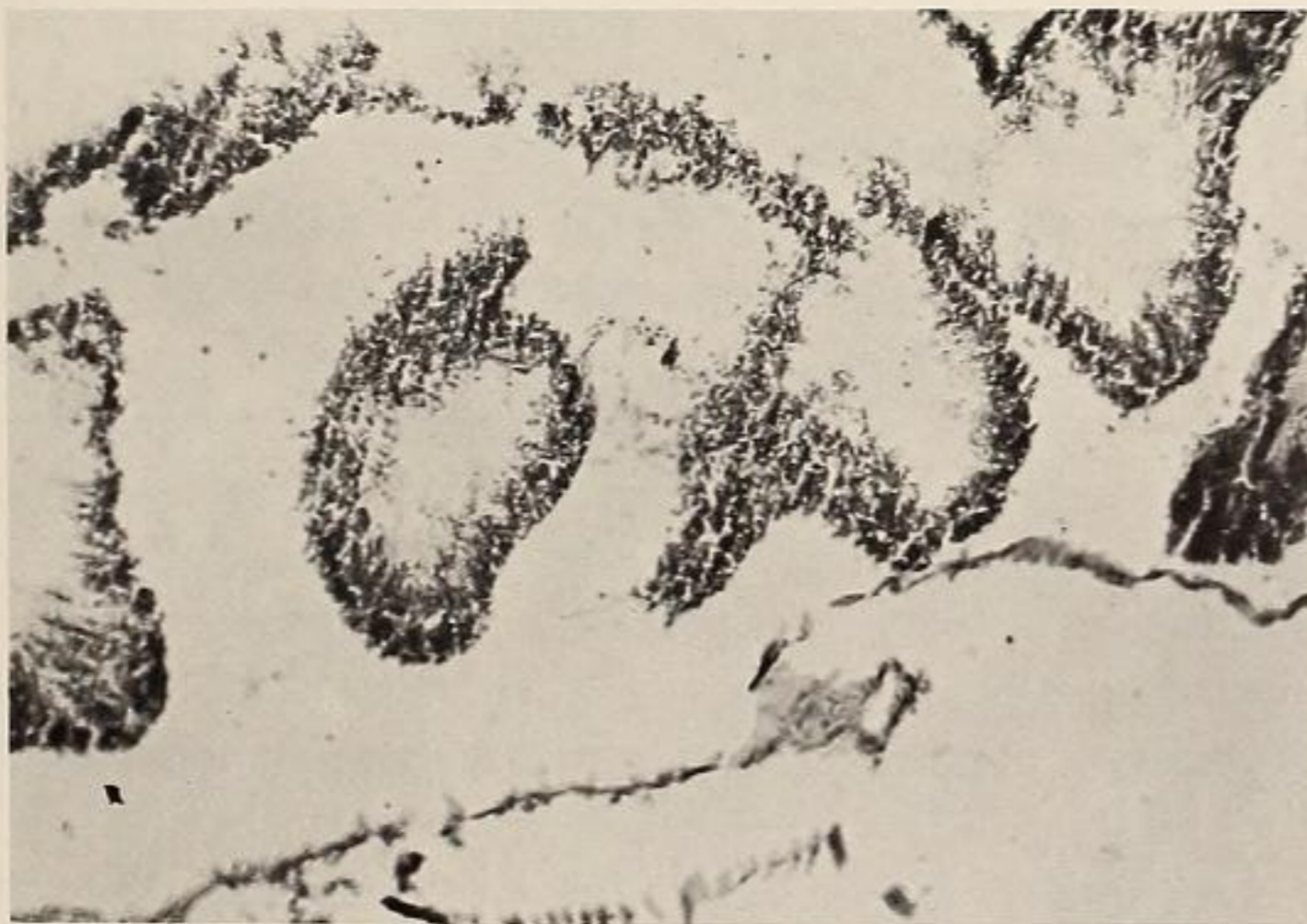


Figure 3

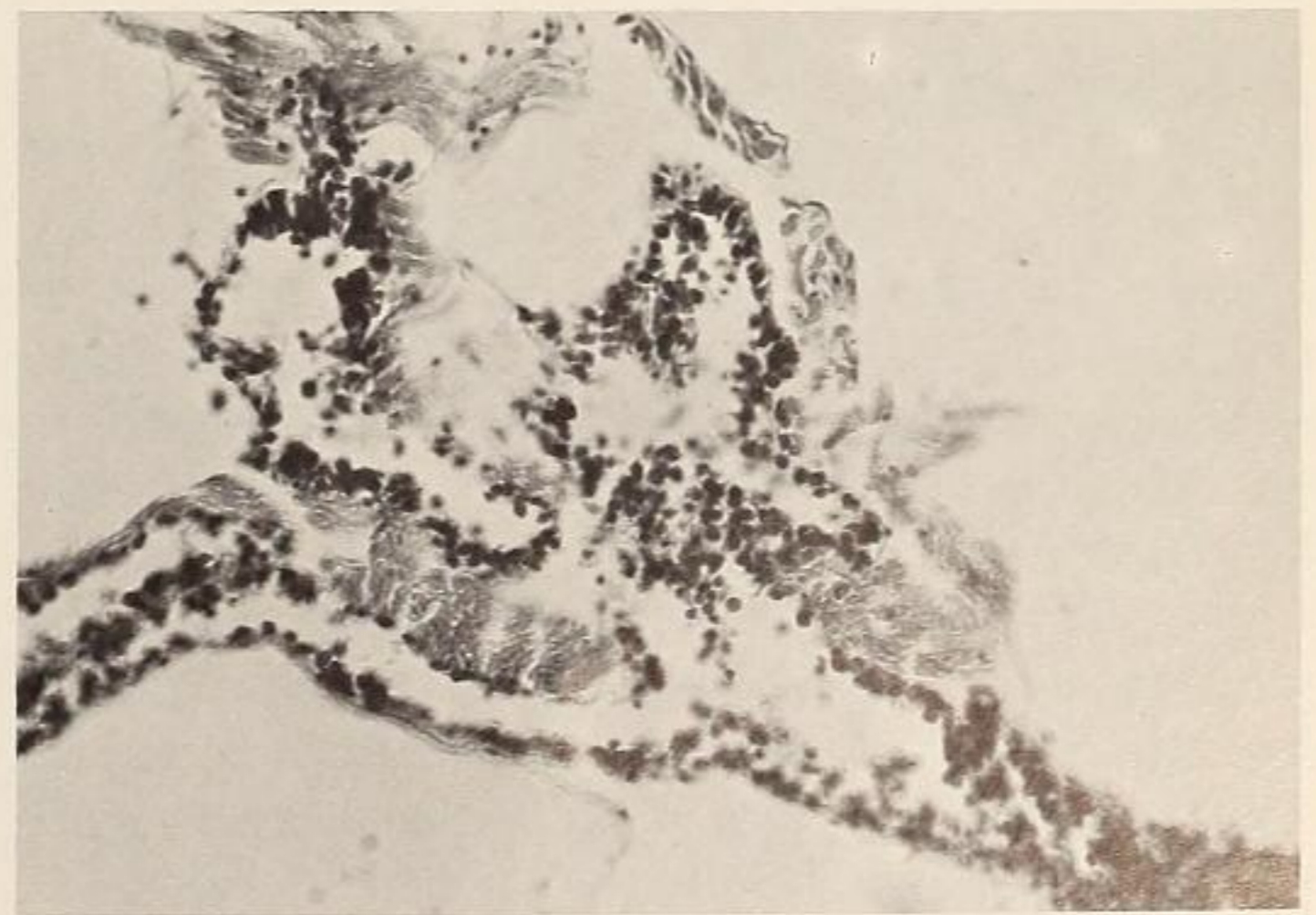
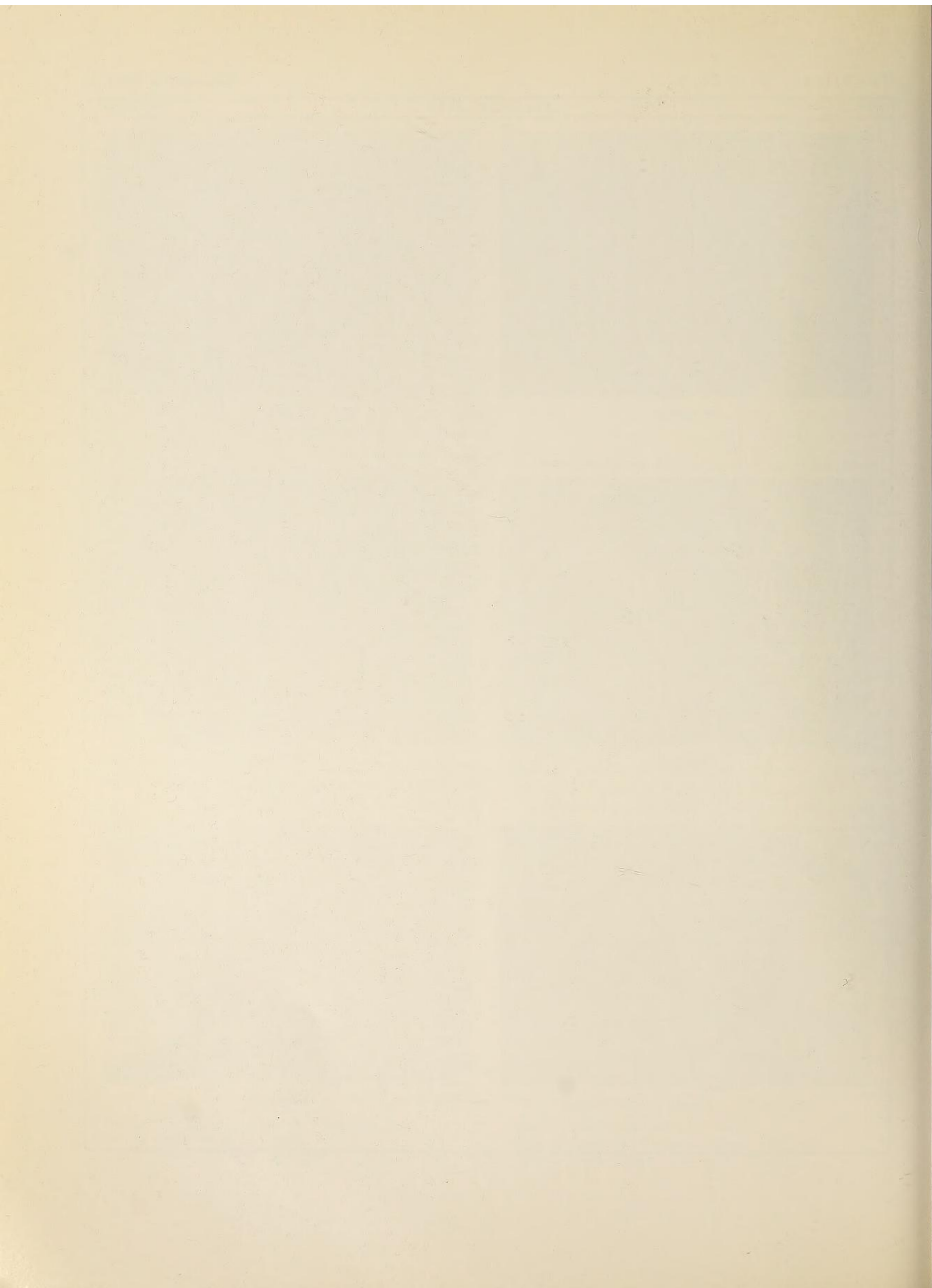


Figure 6



and March, and by April the gametes in 1 of 38 animals sectioned were morphologically ripe.

Experiments with *Mulinia lateralis* made it evident that at least some gametes of this species were both morphologically and physiologically ripe at times other than during the normal spawning period. LOOSANOFF (1937) reported the possibility that the hard-shell clam, *Merccenaria mercenaria* (LINNAEUS, 1758), contained physiologically and morphologically ripe gametes at times other than during the immediate prespawning or spawning period. In one experiment with *Mulinia lateralis*, animals that were collected on January 10 at a water temperature of -0.1°C were brought into the laboratory and both males and females were induced to spawn immediately when placed in water of 20°C . The eggs released were fertilizable, but larval development was poor. In another experiment, animals collected on January 23 at a water temperature of 4°C were immediately induced to spawn in the laboratory. They released eggs that developed into normal straight-hinge larvae within 24 hours after fertilization.

By mid-April the water temperature in the outdoor tanks increased to about 7°C ; gametogenic activity also increased and one ripe female was found. As stated previously, however, some animals had follicles with at least a few morphologically ripe gametes throughout the winter. As the water temperature increased to about 10°C in mid-May, vigorous gametogenesis was resumed and 38.3% of the adult animals examined were ripe. This rapid proliferation and maturation of sex cells continued through June and July. Follicles of ripe males were crowded with spermatozoa whose tails filled the center of the lumina (Plate 37, Figure 2). Each follicle of ripe females contained many mature ova which appeared to be free in the lumen or attached to the follicle walls by slender stalks (Plate 38, Figures 8, 9).

The first partially spawned individuals were in samples taken July 10 to 12, when the water temperature had reached approximately 20°C (Text figure 1). By the end of August 61.3% of the animals examined were partially spawned and a few were spent. Slight gametogenic activity began again in late July and August, followed by rapid proliferation of sex cells in September. More ripe clams were found in September than in August, but it was not determined whether this gametogenic activity produced a second wave of spawning. During the colder months, from October through April, a few individuals appeared to be in the inactive stage (Plate 37, Figure 6; Plate 38, Figure 12). At this stage it was most difficult to distinguish between the sexes. The presence of one or two ova or spermatozoa in some follicles, however, enabled me to sex the animals.

Of a total of 597 *Mulinia lateralis* examined, 302 (50.6 per cent) were females and 295 (49.4 per cent) were males. No hermaphrodites were found. Hermaphroditism is rare in the closely related surf clam, *Spisula solidissima*, and then is apparently a developmental accident (ROPES, 1966, 1968b).

PLANKTON STUDIES

It was possible to study the occurrence of *Mulinia lateralis* larvae in the plankton only during the summer of 1968 when about 350 samples were collected and examined. Larvae first appeared in the plankton on July 8, when the water temperature ranged from 16° to 20°C at the various stations sampled. Since it was impossible to identify positively all bivalve larvae in a sample, exact numbers of *M. lateralis* larvae were not recorded. However, 2000 to 3000 *M. lateralis* larvae per 200-gallon sample were considered abundant and 10 to 20 larvae considered few. Larvae were abundant on July 23 at water temperatures of 19° to 21°C . Numbers were reduced drastically by July 29 and no larvae were found in samples collected August 1 to 8. Larvae were again present on August 12 and were abundant in samples of August 19 to 22. The number of larvae decreased again in late August and by September 3 only a few were in the plankton samples. Numbers continued to be low through October 8, but even on October 24 (when the last samples were collected) the samples contained a few larvae. Water temperature by this time had decreased to about 17°C .

MODE OF REPRODUCTION AND PELAGIC EXISTENCE

The examination of gonad sections suggests several generalizations about the reproductive cycle of *Mulinia lateralis*. Gametogenesis is essentially continuous throughout the year, i. e., there is no completely inactive period in winter or summer. Some individuals at each sampling period showed gametogenic activity, but gametogenesis was most active and more ripe gametes were present from mid-July through August. A spawning peak was reached in August and development of ripe cells again increased in September. It was not determined whether this September development indicates a second reproductive cycle because gametogenesis does not totally cease before this period.

The abundance of larvae in plankton samples disclosed that two peaks of spawning occur; one is in late July, just after spawning begins (as determined from histological sections), and the second and greater one is in middle to

late August, which coincides with the increased percentage of partially spawned animals in my histological preparations (Text figure 1). This second peak of spawning appears to be the major one for the normal reproductive period, as noted by LOOSANOFF *et al.* (1966), who stated that *Mulinia lateralis* larvae were extremely numerous in plankton samples from Long Island Sound during the latter part of the summer. SULLIVAN (1948) found *M. lateralis* larvae from mid-July to September in plankton samples from Malpeque Bay, Prince Edward Island, Canada, a period comparable to that in the present study. Even though Malpeque Bay and Long Island Sound are widely separated geographically, their temperature regimes are similar. SULLIVAN first observed *M. lateralis* larvae in plankton samples about July 18, when the water temperature was about 21° C, and continued to take larvae until early September, after which time the water temperature had decreased to about 17° C. These dates and temperatures correspond closely with the data of the present study. This similarity tends to indicate that these two populations of *M. lateralis*, even though widely separated geographically, are physiologically similar. The temperature requirements for gonad development and spawning of both groups appear to be the same.

SHAW (1965) collected recently set *Mulinia lateralis* larvae in modified Thorson bottle collectors in the Tred Avon River, Maryland, from May into November and reported that this clam appeared to have a continuous period of setting with no apparent gaps during the spawning season. Although he did not report temperature data for this period, I determined from other sources that water temperatures in the Tred Avon River from May to November approximated those in Long Island Sound from July to September. It appears, therefore, that *M. lateralis* from Long Island Sound and the Tred Avon River, Maryland, may have similar temperature requirements for gonad development and spawning.

ROPES & STICKNEY (1965) reviewed the information available on the reproductive cycle of *Mya arenaria* and indicated that two important phenomena occurred in this species: "(1) the tendency for these clams to develop gametes and spawn progressively earlier in the season northward and southward from northern Massachusetts, and (2) the bimodal nature of spawning south of Cape

Cod." They further indicated that the bimodality may not have resulted from two distinct reproductive cycles, but rather from an interruption in the spawning during a single cycle. Bimodality of spawning appears to occur with *Mulinia lateralis* also. Another possible reason for the appearance of high numbers of *Mulinia lateralis* larvae in late summer may be that some of the larvae which had set during the earlier peak had become sexually mature and spawned during the summer. I noted several times during this study that under laboratory or natural conditions little time was required for a new generation of *M. lateralis* to develop gametes. Under natural conditions I determined that these clams develop gametes within 6 weeks after setting. Some animals found in outdoor tanks in late August, which had entered the tanks as free-swimming larvae in early July and then metamorphosed, had grown to sexual maturity. These specimens appeared to be ripe and I was able to spawn them in the laboratory. They may also have been capable of spawning under natural conditions.

The possibility, however, that populations of *Mulinia lateralis* from different areas belong to different physiological races is not excluded. NELSON (1928a, 1928b) was of the opinion that populations of American oysters, *Crassostrea virginica* (GMELIN, 1791), which inhabit the Atlantic Coast and the Gulf of Mexico were composed of individuals of the same physiological race, or variety, showing the same physiological requirements, such as the minimum temperature at which they could begin spawning. Later studies by COE (1934), LOOSANOFF & ENGLE (1942), LOOSANOFF & TOMMERS (1948), STAUBER (1950), and LOOSANOFF & NOMEJKO (1951) suggested the existence of physiologically different groups within the general population of American oysters. LOOSANOFF (1969) studied the gametogenesis of different groups of American oysters from Long Island Sound, New Jersey, Virginia, South Carolina, and Florida by keeping them in Milford Harbor, Connecticut, for about 3 months and then subjecting them to a long conditioning period at temperatures of 12°, 15°, or 18° C. The sharp differences in the rate of development of their gonads caused him to conclude that there are physiologically distinct populations of oysters that require different temperature regimes for completion of gametogenesis and spawning.

Explanation of Plate 38

Section of Gonad Tissue of Female *Mulinia lateralis*

Figure 7: Active phase of oogenesis. (× 500)

Figure 8: Fully mature. (× 200)

Figure 9: Fully mature. (× 500)

Figure 10: Partially spawned. (× 200)

Figure 11: Spent, with few ova retained. (× 200)

Figure 12: Inactive phase. (× 200)

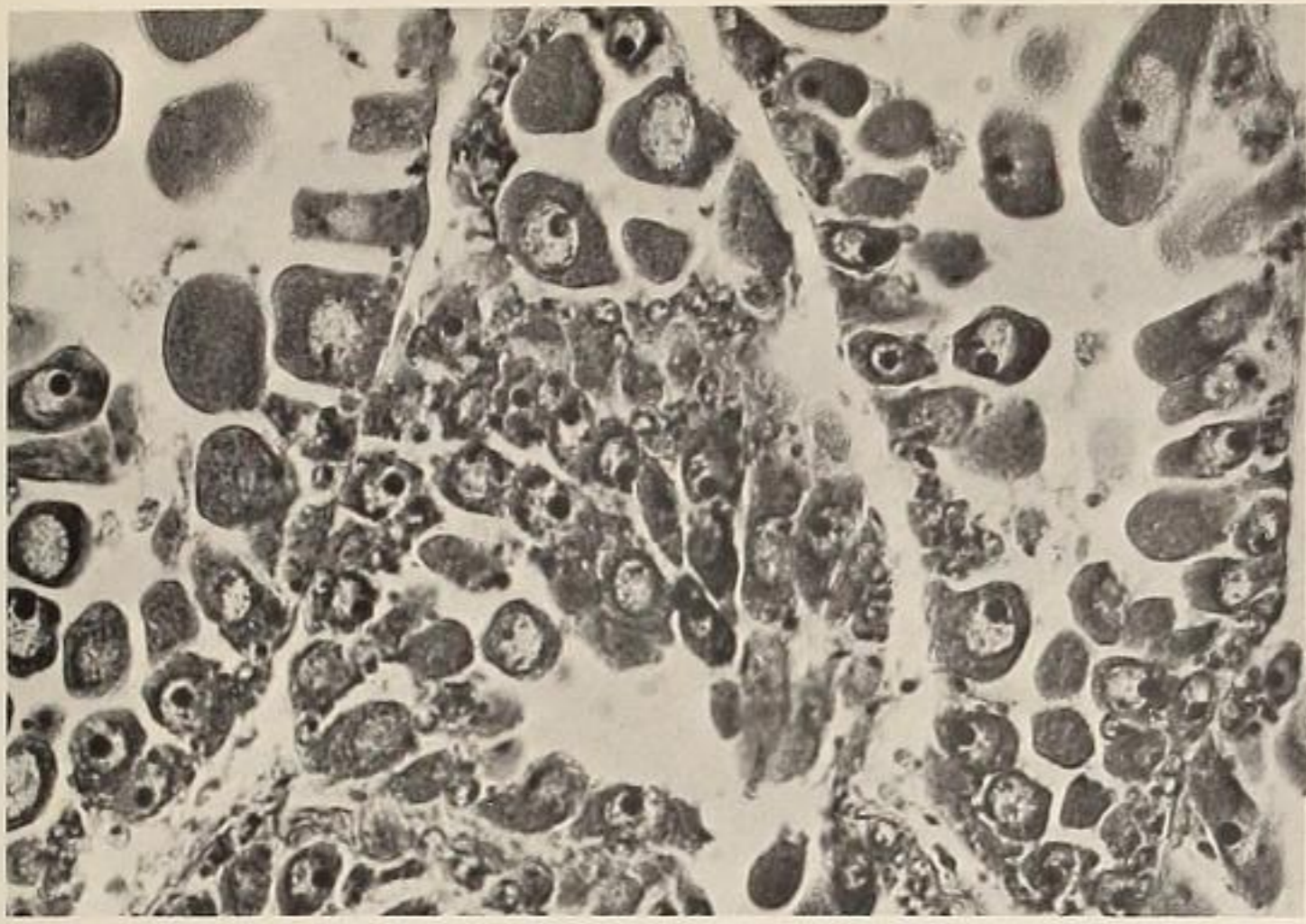


Figure 7

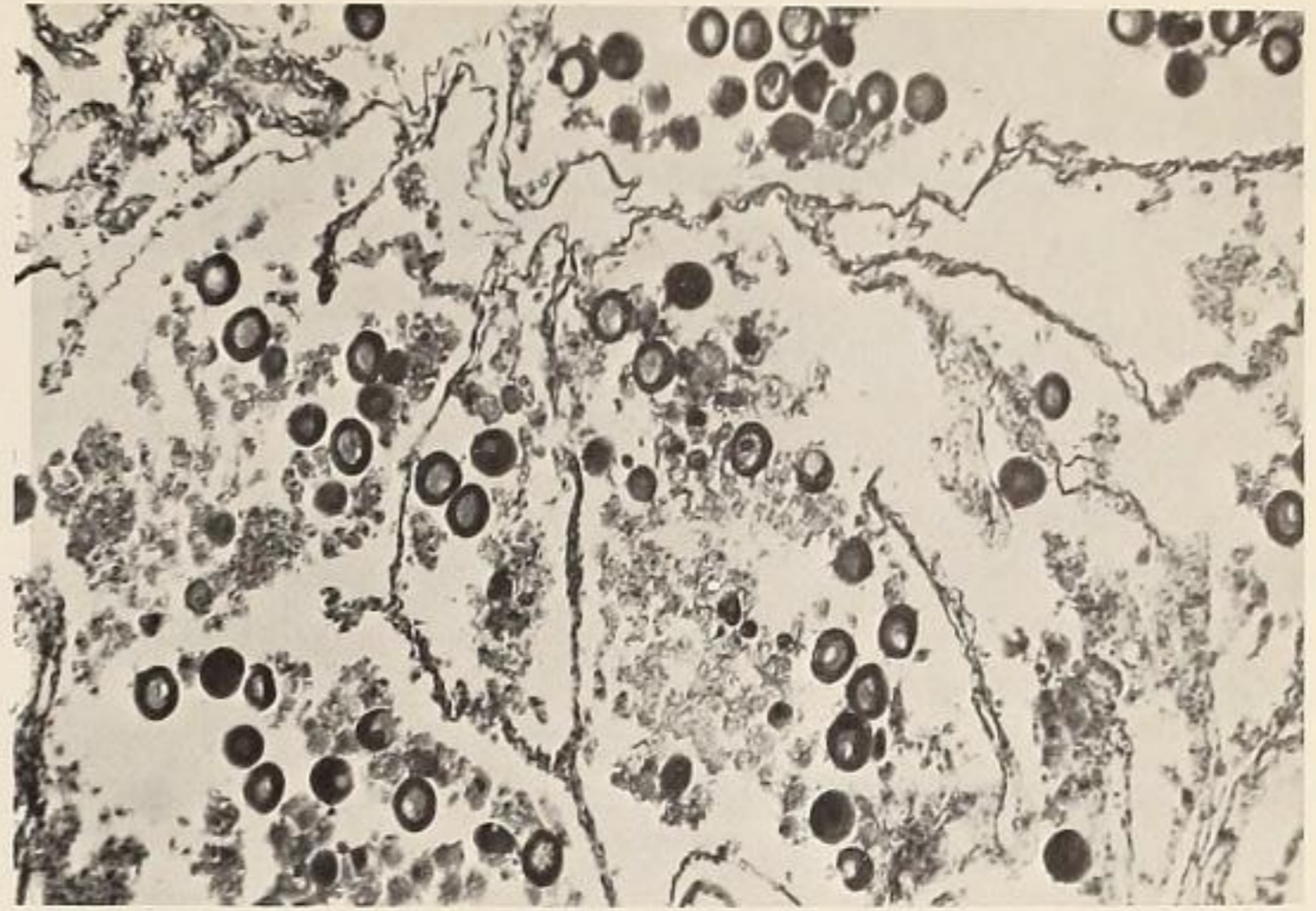


Figure 10

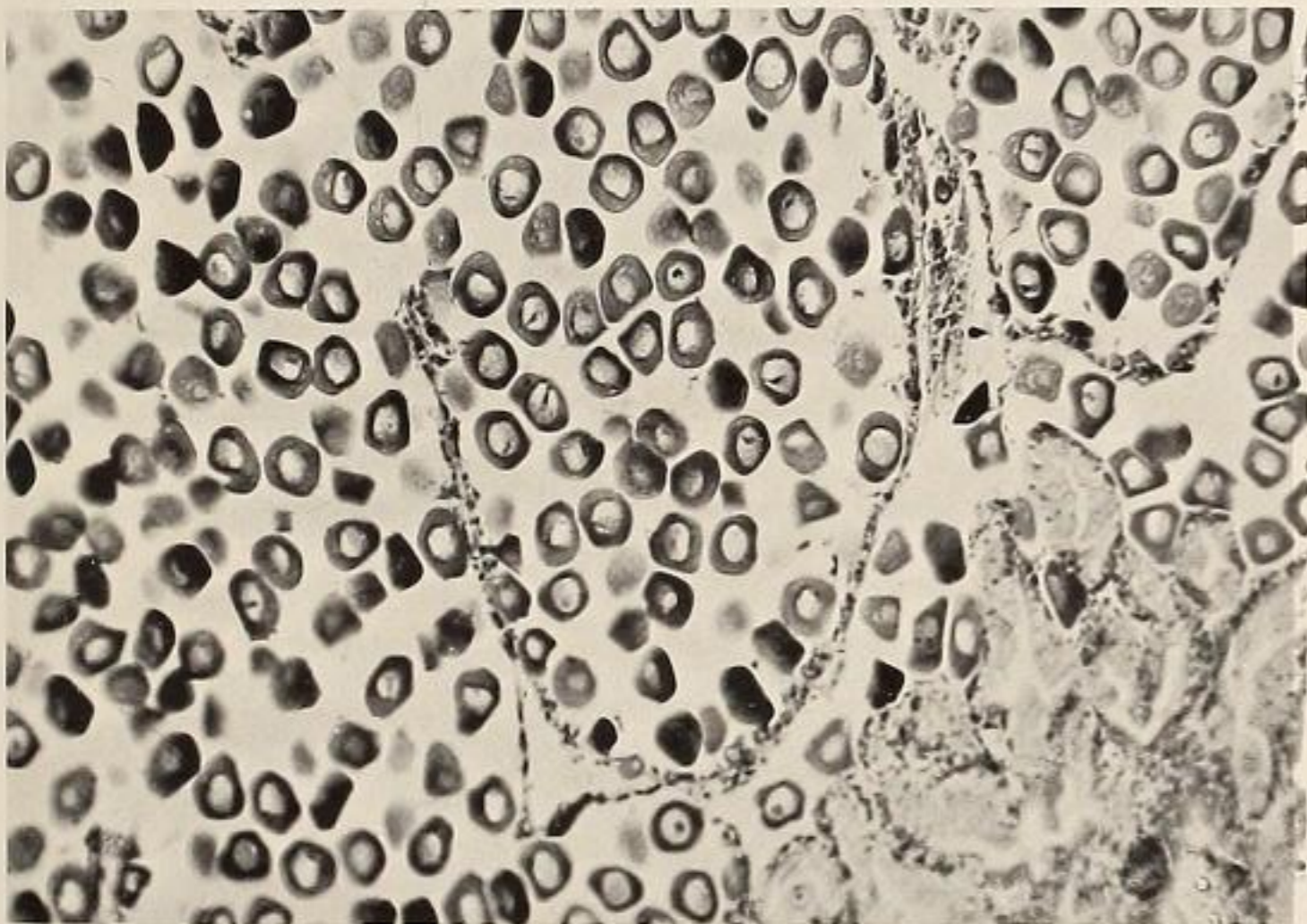


Figure 8



Figure 11

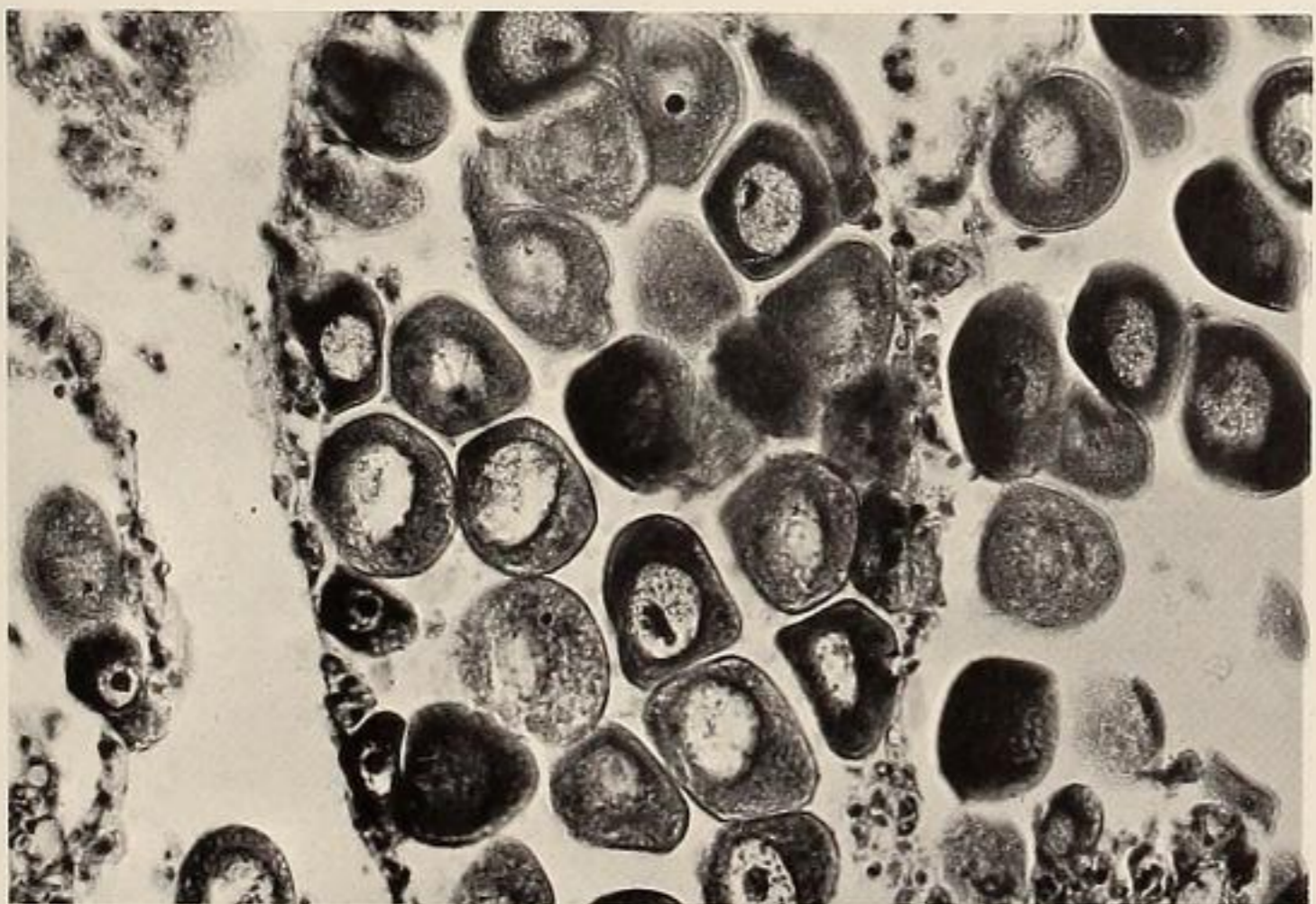


Figure 9

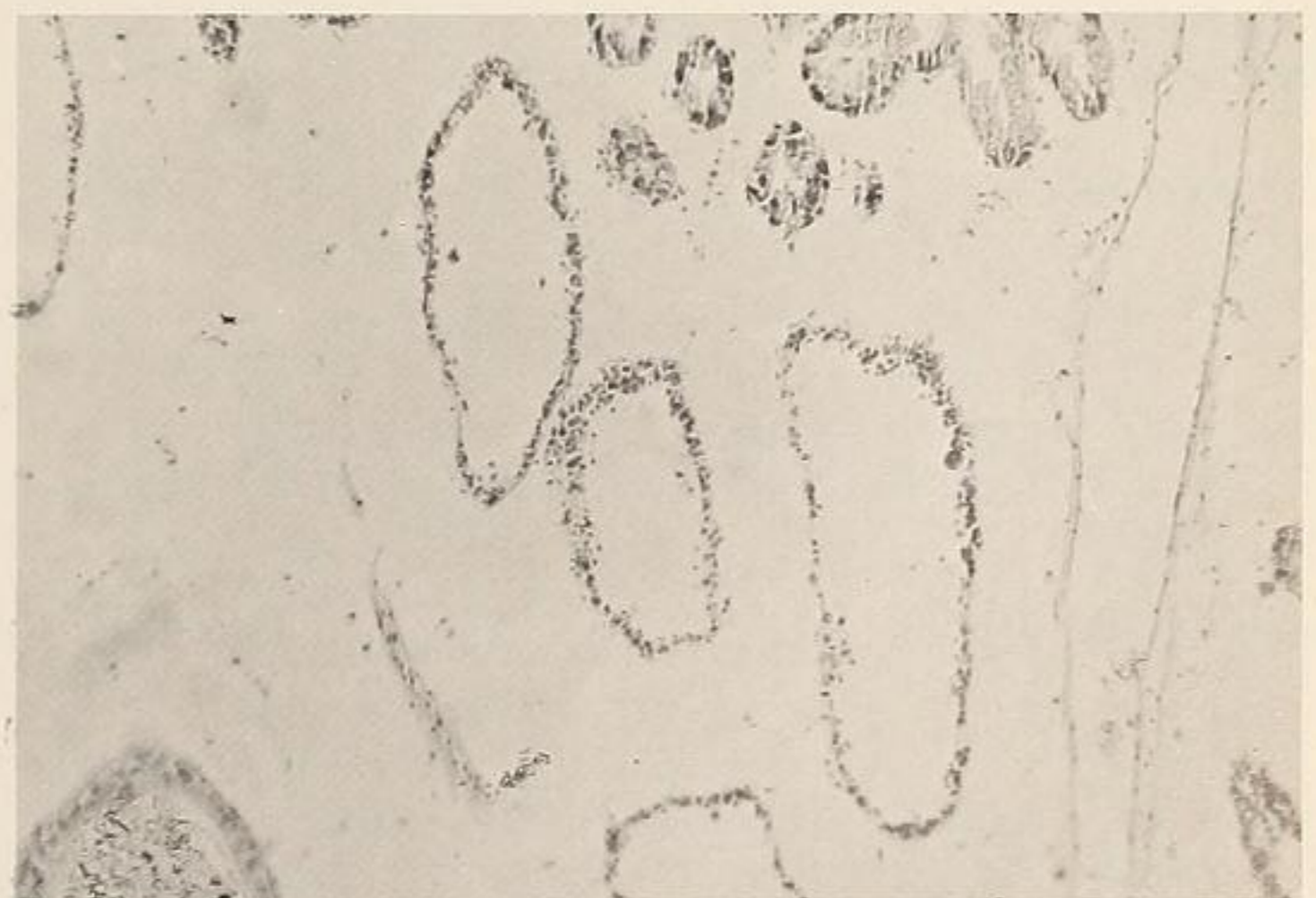
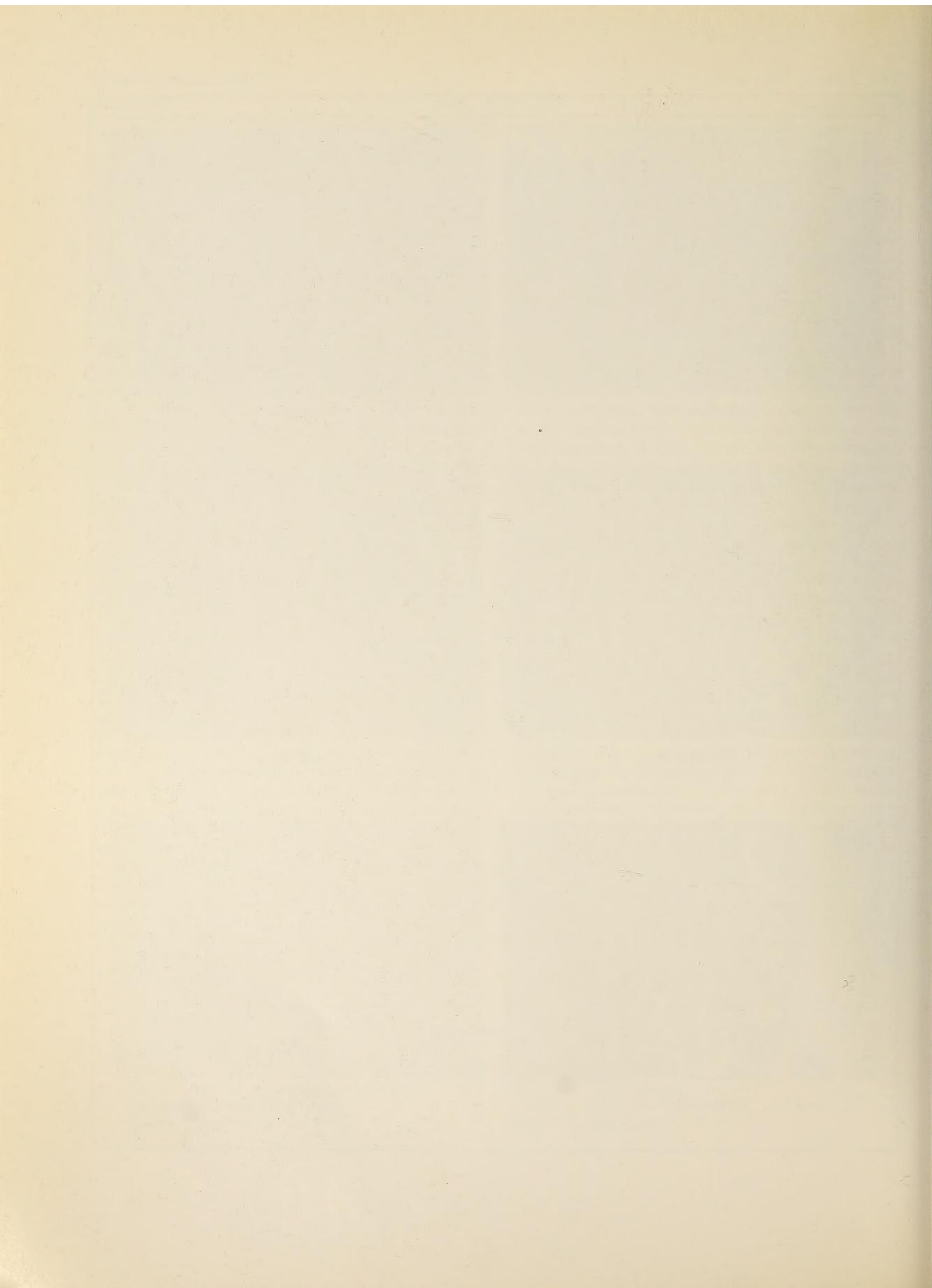


Figure 12



SUMMARY

1. Gametogenesis of *Mulinia lateralis* proceeded throughout the year; the rate was low during October and November, immediately after spawning had occurred, and then increased rapidly in December.
2. Of a total of 597 clams examined histologically, 302 (50.6%) were females and 295 (49.4%) were males. No hermaphrodites were found.
3. It was determined from histological sections that spawning began in early July, when the water temperature increased to approximately 20° C, and continued into September.
4. Larvae first appeared in plankton samples in early July at water temperatures of 16° to 20° C and continued to be in the plankton from early July to late October. Peak abundance was in middle to late August, which coincided with the period when histological samples showed greatest abundance of mature gametes.
5. It is probable that more than one generation of *Mulinia lateralis* is produced in a single year.

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