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## LARVAL CULTURE OF THE CALICO SCALLOP, *ARGOPECTEN GIBBUS*<sup>1, 2</sup>

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### ABSTRACT

Mature calico scallops, *Argopecten gibbus*, collected from the grounds off Cape Kennedy, Florida, were induced to spawn in the laboratory. Fertilized eggs were reared to postlarvae in sea water of  $23^{\circ} \text{C} \pm 2.0^{\circ} \text{C}$  at a salinity of 35 ‰. The external morphology of eggs and developing larval stages are described.

### INTRODUCTION

The calico scallop, *Argopecten gibbus* (Linné), (Fig. 1)<sup>3</sup> is a commercially valuable shellfish which supports a developing fishery off the southeastern coast of the United States and in the Gulf of Mexico. Large concentrations of this benthic marine pelecypod occur on the continental shelf in the area of Cape Kennedy, Florida, in depths from 9-74 m (Drummond, 1969). Concentrations also occur south of Cape Hatteras off North Carolina in depths from ca. 13 m (Bullis and Thompson, 1965) to at least 94 m (Cummins, Rivers and Struhsaker, 1962). The general distribution of this organism is given by Allen and Costello (1972).

The National Marine Fisheries Service (NMFS)

initiated a life history study of calico scallops in 1969. A portion of the study was concerned with the early life history of this mollusk. The purposes of this paper are: (1) to present illustrations of the gross morphology and time sequence of larval development so these stages may be readily identified in plankton samples, and (2) to make available procedures for the mass culturing of this species.

Previous works on larval development of mollusks of the genus *Argopecten*<sup>4</sup> are by Belding (1910), Gutsell (1930) and Sastry (1965). These papers deal with a closely related species, the bay scallop, *Argopecten irradians*.

### MATERIALS AND METHODS

Techniques to induce spawning and rear molluscan larvae suggested by Loosanoff and Davis (1963) were modified at the Virginia Institute of Marine Science (VIMS) in rearing calico scallop larvae. Mature calico scallops (shell width 55 - 65 mm) were collected by otter trawl from the grounds off Cape Kennedy, Florida. They were transported to the NMFS Laboratory in Miami, Florida, in insulated containers of aerated sea water maintained at  $20 - 23^{\circ} \text{C}$ . At the laboratory, scallops were held on water tables and/or troughs of running sea water. Subsequently, a portion

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<sup>2</sup>Contribution No. 478, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

<sup>3</sup>Two terms are used in this paper to define shell dimensions. They are: (1) Length (L), a straight line measurement of the greatest distance between the anterior and the posterior shell margins; (2) Width (W), a straight line measurement of the greatest distance between the umbo and the ventral shell margin. Several authors use the term "height" for the dimension we define as width.

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<sup>4</sup>Waller (1969) rejected the generic name *Aequipekten* and suggested *Argopecten*, the name currently in use.

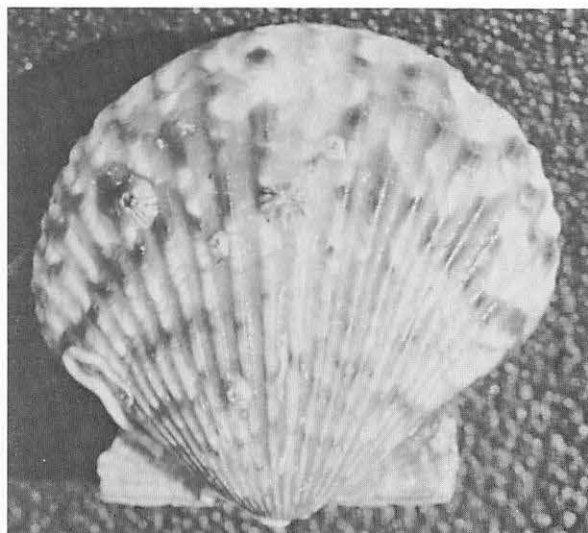


FIG. 1. The right valve of a mature calico scallop, *Argopecten gibbus* - shell width 56 mm.

of these mature scallops was air-shipped to VIMS at Gloucester Point, Virginia, where spawning and larval rearing to setting were accomplished.

All culture techniques and most of the morphology were described from specimens, photomicrographs and information obtained from induced spawning and larval rearing at VIMS.

#### Induction of Spawning

Ovarian color is a reliable index of sexual maturity in calico scallops (Miller, Hudson, Allen and Costello, 1972).<sup>5</sup> Before we attempted to induce spawning, scallops were selected that showed orange-red ("ripe") ovarian color. The ovarian color was easily observed as the scallops gaped in the troughs of running sea water. Preliminary observations indicated that induced spawning in ripe calico scallops is easily achieved. We induced spawning several times in less than one hour by raising the water temperature from ca. 20 - 25° C. To trigger spawning, in addition to raising the water temperature, it was occasionally necessary to strip gametes from one mature calico scallop specimen and, with a pipette, introduce them gently into the water containing gaping scallops.

Calico scallops are hermaphrodites. Sperm cells are

usually extruded first when spawning is induced in the laboratory. After sperm cells have been discharged for 30 min to an hour, discharge of eggs begins. Once spawning begins, it may continue for several hours.

When techniques to induce spawning were established, 10 ripe scallops were selected. Their shells were carefully scrubbed to remove a variety of encrusting invertebrates which are frequently affixed to the outer shell (Wells, Wells and Gray, 1964). If these fouling organisms, e.g., the serpulid polychaete, *Pomatoceros caeruleus*, are not removed, they may spawn when spawning is induced in the scallops and contaminate the larval culture.

After cleaning, the scallops were placed, one to a dish, in 3"x5"x9" Pyrex glass containers, each  $\frac{3}{4}$  filled with filtered 20° C sea water at a salinity of 32.1 ‰. The containers were then placed on a water table. A black cloth was placed between the glass containers and the table top to aid in observing when spawn was first extruded. Temperatures in the dishes containing scallops were raised from 20 - 25° C by flowing warm tap water around them. In two of the dishes, sperm cells stripped from another mature calico scallop were introduced with a pipette. The scallops in these two dishes began to spawn 78 min after the water temperature reached 25° C. Six additional scallops spawned at various intervals in the next hour.

When the water in each dish became clouded (opaque) with suspended sperm, the scallop was removed and placed in a clean dish of 25° C filtered sea water. This procedure was continued until the scallop began to discharge only eggs. The scallop was then placed in a clean dish of 25° C filtered sea water where it was kept until spawning was completed. Dishes containing mixed sperm and eggs were discarded.

Since the eight scallops induced to spawn began extruding sperm and then eggs at various times over ca. a 2-hr period, we had available, simultaneously, dishes containing freshly spawned, unmixed suspensions of sperm cells, and freshly spawned, unmixed suspensions of eggs. A light suspension of sperm (35 cc) was added to each of the dishes containing eggs, and the mixtures were gently agitated. Following fertilization, the eggs were washed in a stainless steel screen (152  $\mu$  openings) to remove debris that accompanies spawning. We followed the washing procedure described by Loosanoff and Davis (1963).

After the fertilized eggs were washed, they were added to a container of filtered sea water and the number of eggs per unit of sea water was determined with a Sedgwick-Rafter cell. A sufficient quantity of

<sup>5</sup> Miller, G. C., J. H. Hudson, D. M. Allen, and T. J. Costello. 1972. Ovarian color changes in calico scallops, *Argopecten gibbus*. Unpublished manuscript filed at the National Marine Fisheries Service, Southeast Fisheries Center, Miami Laboratory, Miami, Fla.

the washed egg suspension was added to a 20-liter container<sup>6</sup> of filtered sea water to provide 25 eggs/ml. This concentration was reduced to ca. 10 larvae/ml at the straight-hinge stage.

Temperature in the culture was maintained at  $23^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$  throughout larval development. To simulate conditions in the calico scallop's natural offshore spawning area, salinity was adjusted to 35 ‰ immediately after fertilization and held at this concentration. The culture was not aerated, and no illumination was provided. Water was changed every other day by straining the entire 20 liters through a stainless steel screen. A screen with mesh openings of  $50\mu$  was used initially; larger openings were used as the larvae increased in size. Larvae retained on the screens were returned to clean 20-liter containers of filtered sea water. Following the first two water changes, 0.2 cc of "twin biotic" (a mixture of streptomycin and penicillin) was added per liter of culture to retard bacterial growth. Feeding of the larvae was initiated 30 hr after fertilization. Unialgal cultures of *Monochrysis lutheri* were fed in quantities sufficient to provide, initially, concentrations of ca. 60,000 cells/ml. As the larvae grew, adjustments to concentrations of food were made to quantities where observations showed complete utilization.

#### EMBRYONIC DEVELOPMENT

Embryonic development of *A. gibbus* is similar to that described by Sastry (1965) for *A. irradians*. A detailed study of early cleavage was not made; therefore, the times that are reported for early embryonic development are approximations based on the most

<sup>6</sup> Plastic garbage can

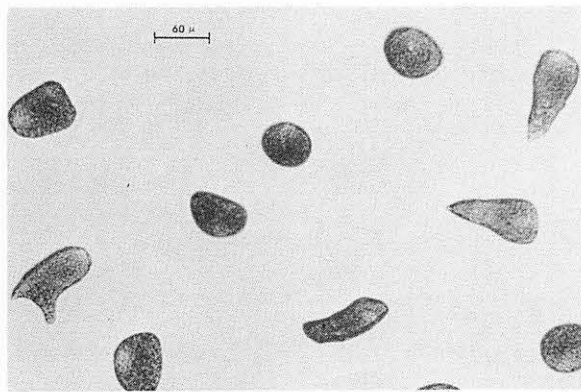


FIG. 2. *Argopecten gibbus* eggs ca. 35 min after spawning. Note irregular shape of most eggs.

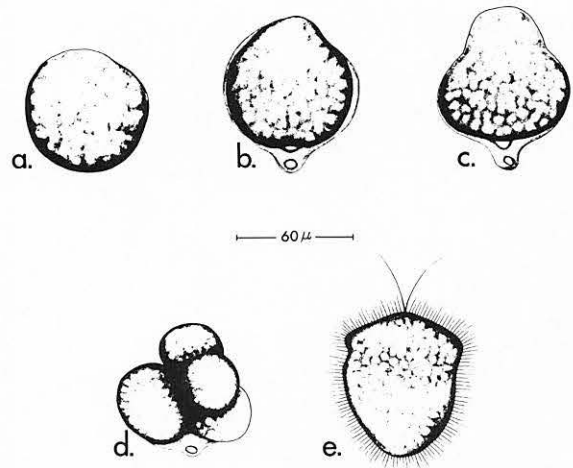


FIG. 3. Embryonic development of *Argopecten gibbus*: a) unfertilized eggs; b & c) zygotes 40-60 min after fertilization showing polar bodies; d) cell division ca. 100 min after fertilization; e) a ciliated trochophore 24 hr after fertilization.

typical stage represented in the culture samples observed. Developing zygotes from a single spawning showed considerable disparity in rates of development during the first 24 - 36 hr. Newly spawned eggs of *A. gibbus* were asymmetrical (Fig. 2), though observations of *A. irradians* eggs observed after spawning also appeared similarly asymmetrical.

Unfertilized eggs, measured with an ocular micrometer, averaged  $60\mu$  in diameter (Fig. 3a). Approximately 40 min after fertilization, two polar bodies formed as the zygote gradually modified to form a polar lobe (Figs. 3b and 3c). In most cultures discernible cleavage began 70 min after fertilization. As in the embryonic development of many other molluscs, unequal blastomeres were noted in all early cleavages, and micromeres proceeded with more rapid division than macromeres during the first 8 hr of development. Figure 3d depicts typical cell division 100 min after fertilization. Active ciliated trochophores were observed 24 hr after fertilization (Fig. 3e).

Shell secretion began during the early trochophore stage. The shell gradually enveloped the body and an active straight-hinge veliger was formed before the larvae were 48 hr old.

#### Larval Culture

Under our laboratory conditions, the larval period of the calico scallop was 16 days. Figure 4 is a composite made from photomicrographs taken every 24 hr. The larvae, items B through J in Figure 4, repre-

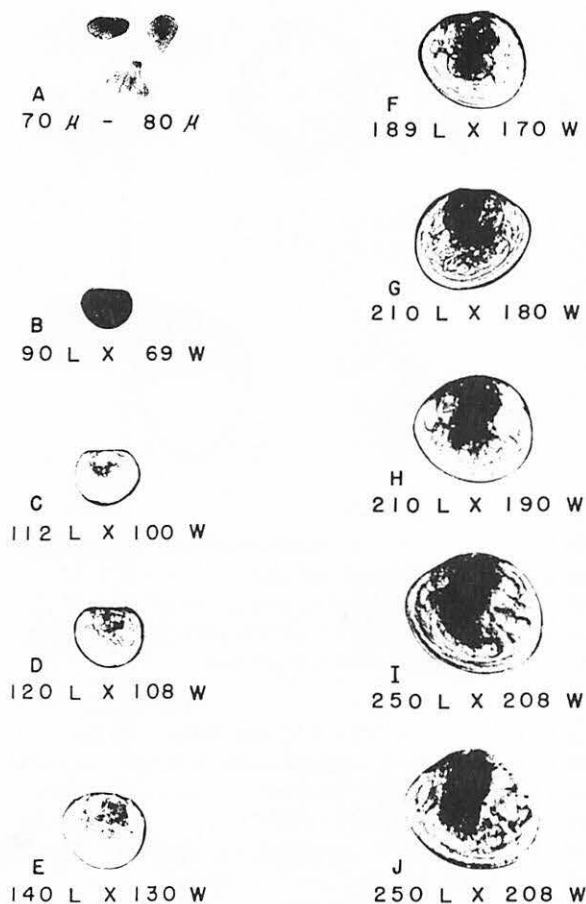


FIG. 4. Composite photomicrograph of larval *Argopecten gibbus*. Age in days: a) 1; b) 2; c) 4; d) 5; e) 7; f) 9; g) 11; h) 13; i) 15; j) 16. Length x width dimensions are given in microns.

sent the average sizes for each time stage obtained by measurement of 25 larvae from several photomicrographs of each 24-hr period. The early straight-hinge larvae appeared to be chopped off at one point along the hinge line. The umbo appeared at about 140 μ, rounded and poorly defined. It remained inconspicuous throughout larval development. Figures 5 and 6 show typical morphological features in the latter stages of larval development and just prior to setting. Chanley and Andrews (1971) made effective use of hinge line shapes in describing 23 species of bivalve larvae. The hinge line shape of the calico scallop larvae (Fig. 7) is distinctive but very similar to *A. irradians*. The toothed area is comprised of three taxodont teeth at each end of the hinge line. The central hinge area is undifferentiated. Other identifying characteristics of calico scallop larvae are their

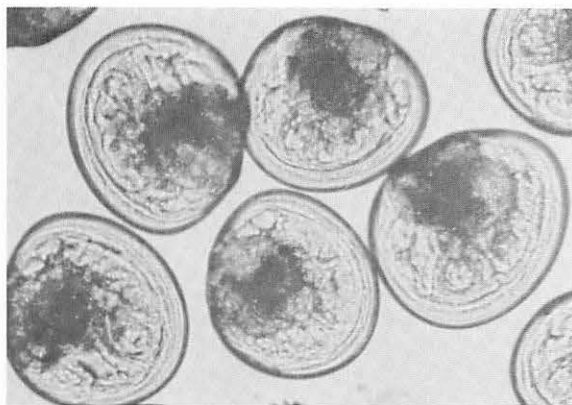


FIG. 5. Photomicrograph of live 12-day-old larvae of *Argopecten gibbus* length 210 μ.

pale color and development of an inconspicuous eyespot when the larvae reach a length of ca. 250 μ.

#### SUMMARY

Calico scallops, *A. gibbus*, have been induced to spawn in the laboratory and the larvae have been reared to setting. Development, on the basis of external morphology, is quite similar to that recorded for a closely related form, *A. irradians* (Sastry, 1965).

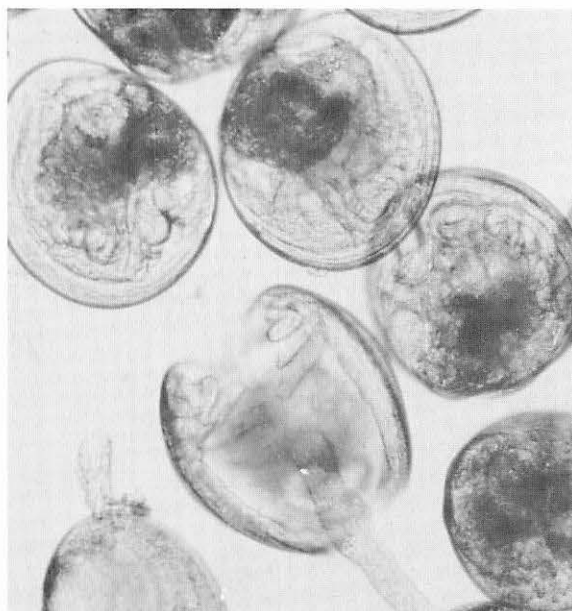


FIG. 6. Photomicrograph of live 16-day-old larvae with foot extended, showing anatomical relationship and early structure of gill and foot.

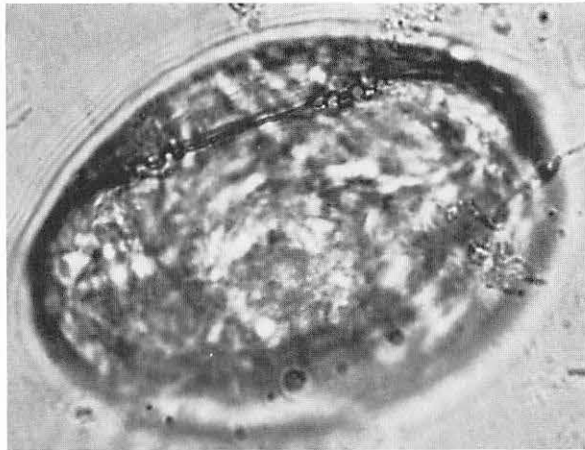


FIG. 7. Dorsal view of hinge of the larval *Argopecten gibbus*.

The major difference is that *A. gibbus* has a much larger pediveliger or newly set larvae which ranges in length from 235-270  $\mu$ . The difference is significant when compared to the bay scallop, *A. irradians*, which sets at a length of from 170-190  $\mu$ .

#### ACKNOWLEDGMENTS

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