NOTE

DEVELOPMENT OF EGGS AND EMBRYOS OF THE SURF CLAM, *SPISULA SOLIDISSIMA*, IN SYNTHETIC SEAWATER

The eggs of the surf clam, *Spisula solidissima*, have been used extensively for investigations of egg structure and embryonic development of bivalves. Allen (1951) has pointed out the advantages of the use of surf clam eggs for research of this nature. These studies have been limited, however, to areas where natural seawater was readily available, due to the unsuitability of most synthetic seawaters for supporting the embryonic development of bivalves (David A. Nelson, NMFS, Milford, Connecticut and Gerald Zaroogian, Environmental Protection Agency Laboratory, West Kingston, Rhode Island, pers. comm.).

Experimental Observations

We recently reared *Spisula solidissima* embryos in a synthetic seawater formulation developed by Zaroogian, Pesch, and Morrison (1969) as a culture medium in which to rear oyster embryos. Our observations were made in salinities of 25 and 30 $^0/_{00}$ at 10°, 15°, and 20°C water temperatures. Within these ranges we found 20°C to be the optimum temperature for development, allowing us to rear eggs to the 5-day-old stage (early veliger) with almost 100% survival and no signs of larval abnormalities. At 20°C polar body formation occurs in about 45 min and the two-cell stage in about 90 min. The early veliger, or straight-hinge stage, is reached in less than 24 h. At 15°C all stages of development are normal but somewhat delayed, with development to the straight-hinge stage requiring more than 24 h. At 10°C the rate of development of all stages is greatly retarded and many abnormal embryos are present. The majority of fertilized eggs held at 10°C requires more than 96 h to develop to the straight-hinge stage.

At 20°C we found that development of fertilized eggs in synthetic seawater was comparable to the best development observed in natural seawater.

This study did not involve testing embryonic development of *S. solidissima* in synthetic seawater over a wide range of salinities, but was limited to those salinities currently in use in other research programs within this laboratory. It appeared that there was no difference in survival and development of eggs to the 5-day-old stage at salinities of 25 and 30 $^0/_{00}$, the only salinities tested. In earlier work, however, Stickney (in Yancey and Welch, 1968) reported that *S. solidissima* eggs failed to develop under experimental conditions in salinities of less than 23 $^0/_{00}$ in natural seawater.

Since the synthetic seawater formulation developed by Zaroogian, Pesch, and Morrison (1969) can be readily prepared, its general acceptance could lead to a wider utilization of surf clam eggs by embryologists and cytologists with standardization of techniques and comparability of results not always possible when natural seawaters from different locations are used.

LaRoche, Eisler, and Tarzwell (1970), in studies of bioassay procedures for oil and oil dispersant toxicity evaluation, suggested the use of Zaroogian's seawater as a standard testing medium in place of natural seawater, the composition of which varies, especially in regard to the presence of trace metals, dissolved organics, and particulate matter. They recommended the use of Zaroogian's seawater because of its ability to support spawning adults and larvae of the American oyster, *Crassostrea virginica*, for at least 48 h without visible adverse effects, and adult mummichog, *Fundulus heteroclitus*, grass shrimp, *Palaemonetes vulgaris*, and sandworm, *Nereis virens*, for extended periods. Thus, when sufficient research has been performed in this area, it may be possible not only to hold adult animals but also to rear the eggs and larvae of these animals in the same synthetic seawater. This would be an obvious advantage in assessing comparative tolerances to pollutants of different life stages.

Collection and Maintenance of Surf Clams in the Laboratory

Although some information on the collection and maintenance of surf clams in the laboratory and their reproductive cycle has been published (Loosanoff and Davis, 1963; Ropes, 1968; Yancey and Welch, 1968), we feel it pertinent to this paper that it be reviewed and our own observations added.

Adult surf clams can be purchased from biological supply houses or collected in their natural habitat. The range of *S. solidissima* is along the Atlantic Coast of North America, from the Gulf of St. Lawrence to Cape Hatteras (Yancey and Welch, 1968). South of Cape Hatteras the surf clam is represented by *Spisula solidissima raveneli*, similar to *S. solidissima* but a smaller species. *S. solidissima* is found in sandy bottoms from the low-tide line to depths of 500 ft in waters of oceanic salinity. They are present in shallow water beds, at various points along their range, and are easily hand-gathered along the coasts of Delaware, New Jersey, Long Island (New York), Rhode Island, and Massachusetts. Our collections have come mainly from Little Narragansett Bay and the area of Point Judith, in Rhode Island.

Surf clams can also be obtained from commercial clam boats working the beds, but our observations have shown that hand-gathered clams are more suitable for laboratory purposes; those obtained from commercial sources are often damaged by the action of the hydraulic dredge used in harvesting. They suffer high mortalities soon after introduction into the laboratory and long-term survival of those remaining also seems inferior to that of hand-gathered stocks.

We feel that the best working size for laboratory animals to be used as a source of gametes is 4 to 5 inches. Larger ones require more space and do not survive as well in crowded tanks. Smaller animals are more difficult to spawn, even though we have found some specimens as small as 5 cm to have viable sex products.

Ropes (1968), in a study of the reproductive cycle of offshore surf clam populations, found a biannual cycle during 3 years of the 4-year period covered by his study. This biannual cycle was characterized by a major mid-year spawning and a minor late-year spawning. He found ripe clams as early as May and as late as October during 3 years of the study. This pattern of ripeness may vary between inshore and offshore populations, depending on local temperature conditions. We found ripe clams only from June to August in inshore Rhode Island waters.

Surf clams can be collected prior to their natural spawning period and conditioned to ripeness in the laboratory. Conditioning refers to a procedure of gradually raising the water temperatures at which bivalves are maintained as a means of achieving gonad ripeness prior to the time one would expect to find ripe animals in the field (Loosanoff, 1954).

We have collected animals with unripe gametes from early winter through late spring (December to May) and conditioned them at 15°C. This temperature equals or exceeds that at which gametogenesis takes place in natural populations (Ropes, 1968). Such animals collected in early winter and conditioned in the laboratory have been spawned as early as March.

Ripe surf clams held in the laboratory at 15°C have never spawned spontaneously; thus, the spawning threshold of this animal in the laboratory would appear to be higher than 15°C. We do feel, however, even though we, as yet, lack quantitative data to substantiate it, that ripe animals held at 15°C tend to resorb their gametes more quickly than those held at a lower temperature following conditioning. Ripe animals collected in June and held at 10°C contained viable sex products in December.

Ropes (1968) reported that offshore populations spawn at lower temperatures than we found in our laboratory populations. He also noted that abrupt rises in water temperature were not clearly a cause of spawning in natural populations. A rapid increase in temperature is certainly an important factor in stimulating spawning in the laboratory. Clams conditioned at 15°C spawned when the temperature was raised quickly to 18-20°C. However, these clams were less responsive than those held in damp refrigeration (approximately 2°C, covered with a wet towel to prevent drying) overnight prior to exposure to 18-20°C. Refrigerated clams normally spawned within an hour after exposure to 18-20°C, while those conditioned at 15°C and exposed to water at 18-20°C did not.

Eggs and sperm can also be obtained by stripping the sex products from the gonads using a

method described by Costello et al. (1957). This involves removing one shell and gill, exposing the visceral mass, and slicing into the gonad which overlays the digestive glands and viscera. Care should be taken to avoid cutting into the underlying intestines and digestive glands, as the presence of body fluids in the cultures of eggs appears to be detrimental to embryonic development. The eggs and sperm are washed into separate collecting containers. Most of the tissue and debris collected along with the gametes can be removed by selective screening.

Stripped eggs tend to be more irregular in shape than naturally spawned eggs but soon become spherical. Previous investigators (Loosanoff and Davis, 1963) have reported the diameter of spawned mature eggs to average 56.5 μ. Our measurements of rounded stripped eggs from ripe clams have agreed with this.

To fertilize the eggs a small quantity of sperm suspension is added to the egg suspension and mixed by rapid stirring; care must be taken to add only a small quantity of sperm as *Spisula* eggs are quite susceptible to polyspermy at high sperm concentrations (Allen, 1951). Following fertilization the germinal vesicle breaks down and a thin membrane forms a short distance above the surface of the egg.

In conclusion we would like to point out that this is the first time to our knowledge that *Spisula solidissima* embryos have been reared in synthetic seawater, although they have been previously reared in the laboratory in natural seawater. Not all synthetic seawaters currently available are suitable for this purpose but that developed by Zaroogian, Pesch, and Morrison has consistently given us good results. We feel that the ability to rear these embryos in synthetic seawater will enhance the value of surf clam eggs and embryos in embryological and cytological research by offering a standardized rearing medium and a comparability of results not always possible when natural seawaters from different locations are used, as well as making possible the use of these organisms in bioassay procedures where the composition of the seawater must be known.

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