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Methods of Hatching Eggs of the Blue Crab¹

MARGARET S. LOCHHEAD AND CURTIS L. NEWCOMBE

INTRODUCTION

The blue crab, *Callinectes sapidus* Rathbun, is the only important marketable crustacean in Chesapeake Bay. While this body of water may be regarded as a center of its numerical distribution, blue crabs in the United States range from Cape Cod south to Texas. Their economic importance is indicated by records of the Federal Government which report for the four-year period 1936-39, an annual average of over 82 million hard crabs valued at about \$526,000 from Virginia and 56 million worth about \$382,000 from Maryland. Soft crab catches in the two states during this period were approximately the same, amounting in each case to over 10 million crabs per year valued at about \$210,000. The commercial value of this fishery, shared by Maryland and Virginia, to local tidewater communities warrants careful examination of the economic and production trends in their relation to sound conservation practice.

In view of the need for information on the early development of the blue crab, studies were begun at the Virginia Fisheries Laboratory in 1940. An effort was made to develop a hatching technic for crab eggs under laboratory conditions that might open the way for large scale application under natural conditions. During the summer of 1941, the crab work was extended and intensified in view of reports of a serious shortage of soft crabs, particularly in Maryland. Aiming to answer questions of practical value to the industry and to crab conservation, studies on the hatching of eggs and experiments on water conditions as they affect hatching and survival of crab larvae were stressed.

Egg bearing or "sponge" crabs predominate during summer in the waters of the lower Bay. Large quantities of egg masses or "sponges" are destroyed when these crabs are steamed in commercial houses. This loss of live eggs has raised the question of whether or not a way may be found to *detach* the egg masses from the crabs when they are landed at the crab house, transfer them to the laboratory, hatch them out there and liberate the larvae to local waters, thus reducing a present waste. The specific objectives involved in this undertaking are—firstly, to develop a satisfactory technic for removing the "sponges" from the crab and transporting them from the commercial crab house to the laboratory; secondly, to find a method of keeping these egg masses

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in a normal condition during varying periods in the laboratory until facilities are available for hatching them; thirdly, to develop a technic for removing small numbers of eggs from the "sponge" preparatory to actual hatching; fourthly, to discover the technics and environmental conditions that are needed for hatching the eggs to the first true zoeal stage; fifthly, to rear first zoeal stage larvae through a series of moults; and sixthly, to find a suitable method for liberating the hatched larvae to waters of the Bay so as to assure survival.

It is the opinion of some investigators that such attempts are not likely to prove fruitful. Needless to say there are many pitfalls to be overcome and an ultimate goal of rearing large quantities of young crabs through all the larval stages preparatory to their liberation in the Bay waters is admittedly not yet in sight. However, the liberation of early zoeal stages hatched from detached "sponges" of commercial crabs is now no longer a problem. The practical benefits likely to accrue from the conversion of an otherwise wasted "sponge" into thousands of larval crabs is not without significance. Expectedly, many will be devoured by predators but this is a natural phenomenon that goes on whether the larvae have a natural or an artificial origin. Presumably, the older the stage in which vigorous larvae are liberated the better their chance of survival but this is by no means a certainty. The success reported by Barnes (1939) in hatching the eggs and rearing the young of lobsters in New England lends encouragement to crab hatching experiments.

An attempt to develop a technic for successful hatching of blue crab eggs on a large scale was undertaken for a second reason, namely, to permit experiments designed to tell under what natural conditions of environment, particularly salinity, eggs may be expected to hatch. In other words, will the eggs of "sponge" crabs occurring off Mobjack Bay or Buckroe Beach hatch out under the salinity conditions that exist there, or do these crabs migrate to the higher salinity waters of the Capes for hatching purposes, or do they simply remain and the eggs fail to hatch out normally? The question of migration may be answered through tagging experiments. Answers to the other questions may be obtained in large measure by carefully controlled hatching experiments. This information is expected to aid in the selection of proper boundaries for crab sanctuaries. For example, if it is found that "sponge" crabs do not undergo a significant migration to the saltier waters of the Capes (a question on which there still seems to be some doubt) then whether they should or should not be protected in areas of less saline water away from the Capes depends on whether or not the eggs will hatch successfully in these areas. These and related problems have important bearing on some of the practical aspects of crab conservation.

There is little, if any, information in the literature on the hatching of blue crab eggs detached from the parent. Churchill (1921), working at Hampton, Virginia during 1916-17, recorded that two "sponge" crabs kept on floats hatched their eggs. Later, Truitt (1939, p. 15) also reported that under laboratory conditions eggs developed and hatched when attached to the mother, but that it was impossible to hatch out eggs which were detached from the parent. This paper presents this Laboratory's findings to date on how successful hatching of detached eggs may be achieved under laboratory and field conditions and also how moulting of the first true zoeal stage to the second instar was obtained.

REVIEW OF LIFE HISTORY

A brief review of existing information on the biology of the blue crab follows. In the less saline waters of the Bay proper and the estuarine waters of the numerous rivers flowing into the Bay, the crabs mature sexually. Here, they mate during the summer at which time the spermatozoa received by the female are stored in special receptacles until the following spring and summer, when the eggs are laid. Whereas the males largely remain in the headwaters, the females start a southern migration reaching the more saline waters of the lower Bay and being ready to lay their eggs by the following spring (Churchill, 1921). The eggs leaving the ovary pass through the seminal vesicles where impregnation by the stored spermatozoa probably takes place and pass down the oviducts to the outside. On leaving the oviducal openings, the eggs become covered with a transparent shell and are cemented to the hairs of the pleopods forming a large egg mass under the abdomen known locally as the "sponge". The "sponge" has been variously estimated to contain from 1,700,000 to 4,000,000 eggs (Smith, 1885; Paulmier, 1903; Churchill, 1921). Churchill's observations on two captive crabs indicate that the incubation period is about two weeks. The life cycle is completed when the young that develop from these eggs undergo successive moults and migrate toward less saline waters of the rivers and upper Bay reaching maturity the following spring and summer at an age of about one year.

MATERIALS

"Sponges" used in the experiments reported here were obtained from fresh crabs in the packing houses at Hampton and Seaford and also from those caught in the York River. They were transported to the laboratory either while attached to the mother crab or in a detached condition. The color of the "sponges" in nature provides a general indication of time of hatching. During the first part of the incubation period, the

"sponge" is yellow in color whereas about five days before hatching-time the eggs darken and finally become dark brown. This is due to changes in their embryonic development. If the embryo moves vigorously and the eyes have developed distinct ommatidia, the eggs are ready to hatch in about a day. When the eggs are within about five days of hatching, it is possible to estimate the hatching time with an accuracy of twenty-four hours.

Uniformity in the developmental stages of the embryos of a "sponge" seems to be a rule. Miss Rosalie Rogers, who assisted in this work, made counts on sixteen different "sponges" and found that only one to four per cent of the eggs of an entire "sponge" were in retarded or undeveloped stages. Eggs taken from the outside and the inside of the "sponge" gave similar hatching results.

Most hatching experiments were carried out in York River water of a salinity varying from nineteen to twenty-one parts per thousand. The temperature of the water in the hatching containers usually ranged from 24 to 27° C. On July 28, 31 and August 10 the temperature was as high as 31° C. When running water was used, the temperature remained around 20° C.

TECHNICS AND EXPERIMENTS

Technic of Transportation. As soon as the fishermen brought their catches into the packing houses, active "sponge" crabs were selected and transferred to the laboratory in containers without water. Best results were obtained by transporting detached "sponges" in jars protected from sunlight and not containing water. When the air temperature was above 30° C. and the period of transportation was two hours or more, the containers were surrounded by a layer of ice.

Technic of Removing Eggs. In removing the whole egg mass, the following method was used. Each pleopod was cut off at the base with a pair of scissors, care being taken to hold the crab so that escaping blood which clots quickly would not cover the eggs and prevent their hatching. The detached sponge remains as a unit and as such may be taken to the laboratory and the eggs removed to hatching jars as described below. Instead of transporting the sponge as a single unit, it may be divided into eight parts by pulling apart the eight pleopods thus providing better aeration and favoring higher survival during transportation to the laboratory.

Two methods of removing the eggs from the sponge mass to the hatching jars in the laboratory were tried. One consisted of removing individual fine strands, 10 to 20 mm. in length, bearing eggs, by means of needles and forceps. These strands were then placed in the hatching jars in the desired quantity. A second

rather crude method, that nevertheless yielded good results, was to slice off of the sponge with scissors an extremely thin section leaving a narrow line of eggs on the blade. On submerging the blade in the water of the hatching jar, the eggs fall off and sink to the bottom. Care should be exercised to keep the strands well separated and to prevent a close grouping of the individual eggs that fall from the blades of the scissors. This method was quicker and hence more commonly used. Thus far, no attempt has been made to hatch out all the eggs of a particular sponge but no special difficulty is anticipated. Experience has shown that some eggs are injured in both methods.

Hatching in Aquarium. The eight pleopods of a "sponge" with eggs attached were tied together loosely and suspended in the middle of an aquarium 32 cm. wide, 60 cm. long and 30 cm. deep. Air bubbles and jets of circulating sea-water penetrated the inside of the egg mass stirring the eggs constantly. Many of the eggs hatched, almost all of which were in incompletely developed stages. During the process of hatching, decomposition set in and the larvae died before reaching the first zoeal stage (Table I).

Hatching in Tall Jar. The use of tall jars with water depth of about eight cm. gave a ninety per cent hatching result only if an average concentration of about 8 eggs per square centimeter of bottom surface was used. Increasing the water depth did not permit the use of more eggs. As soon as the number of eggs was increased the hatching per cent decreased. Eggs in heaps of over three millimeters depth failed to hatch.

Hatching in Plunger Jar. By using the plunger-jar mechanism (modified after Harvey, 1928), the highest hatching percentage achieved was sixty. For a three liter capacity jar one pleopod with attached eggs, i.e. one-eighth of the entire "sponge", was used. The pleopod was cut in from two to four parts and each part attached by threads to the disk of the plunger. At the time of hatching decomposition set in and many of the larvae were in incompletely developed stages and soon died. These conditions were not improved by using running water and additional aeration.

Hatching in Shallow Pan. White enamel pans about 20 centimeters wide and 26 centimeters long were found to be the most satisfactory for hatching the eggs (Table I). The depth of water in the hatching dishes varied from one to six centimeters. Eggs numbering about eight per square centimeter of surface on strands, from one to five mm. long, were placed in the containers. If the eggs were within one or two days of hatching the dishes were often left uncovered. In the beginning, the hatching results were variable due to faulty technics. Best percentages (80-90) were obtained in August when an improvement in the method was worked out.

If several days were required for hatching, either the containers were covered or the sea-water was exchanged every second day. This method was used successfully with eggs ready to hatch in ten or fourteen days. Due to limitations in space, equipment and time few experiments were conducted with such eggs and hatching percentages were not determined.

The shallow pan method of hatching the eggs is simple, gives high hatching percentages and yields vigorous larvae. However, the relative number of eggs which could be hatched in any given container was small. When the number of eggs in the hatching container was increased, decomposition set in, the hatching percentage decreased and the zoeae emerged in incompletely developed stages. It was found repeatedly that by using an average of about eight eggs per square centimeter of surface a hatching percentage as high as 90 may be regularly obtained.

TABLE I
SUMMARY OF EXPERIMENTAL RESULTS ON HATCHING
OF CRAB EGGS

Container	Approximate number of eggs used in individual containers	Highest hatching percentage obtained	Number of experiments	Date of start of experiments
Aquarium 32 x 60 x 30 cm. (water depth)	about two millions	5	3	July 15, 20 and 23.
Plunger jars dia.—12 to 17 cm. water depth—15 to 20 cm.	about 200,000	60	17	July 3, 8, 10, 19, and 24.
Jars dia.—12 to 17 cm. water depth—15 to 20 cm.	100,000 to 200,000	10	18	July 7, 10, 24, 29 Aug. 2 and 4.
Jars dia.—12 to 17 cm. water depth—15 to 20 cm.	1,800	90	18	July 7, 10, 24, 29, Aug. 2 and 4.
Shallow Pans July experiments 26 by 20 cm., water depth—1 to 6 cm.	50,000 to 3,500	35	60	July 3, 23, 24, 28, 29, and 30.
Shallow Pans August experiments 26 by 20 cm., water depth—1 to 6 cm.	3,500	90	60	July 31, Aug. 1, 2, 5, 11, and Sept. 5.

Hatching in the York River. A preliminary experiment was carried out on the hatching of eggs in the York River. The pleopods with eggs attached were fastened in a box made of wire mosquito netting. The box was then suspended in the York

River at Yorktown. These eggs were ready to hatch within twenty-four hours when placed in the River. The following day the eggs were taken to the laboratory and examined microscopically. Examination showed that many of the eggs had hatched and that many were still unhatched but contained living embryos. The hatched eggs showed that in many cases the inner egg membrane was left attached to the outer shell. According to our observations this can be taken as a fairly definite indication that the larvae hatched in a healthy condition. This experiment was carried out on September fifth. Lack of "sponge" crabs after this date prevented the continuation of these experiments.

Development of Larvae. While the experiments on rearing of crab larvae are not germane to the main thesis of this paper, it may be pointed out that a moulting of the first true zoeal stage was accomplished. Numerous attempts were made without success using a wide variety of foods and environmental conditions. Finally, by selecting as food a particular and as yet unidentified dinoflagellate extremely abundant periodically in York River waters during summer, it was possible to obtain moulting of the first zoeal stage.

Various Observations Connected with Hatching. There seems to be no particular time of day when the larvae emerge. We observed hatching at various hours during day and night.

The majority of the eggs of a given sponge hatched out uniformly within twenty-four hours, a few requiring additional time. In some instances new larvae were obtained three days after hatching had commenced, but they were found to succumb soon. Larvae which are healthy at the time of hatching, shed the inner egg membrane and the prezoal skin within a relatively short time. When conditions are unfavorable they fail to do this and may remain enclosed in the prezoal skin for some hours or until they die.

In an attempt to find a means of keeping the egg masses in a normal condition during varying periods in the laboratory until facilities are available for hatching, the eggs were subjected to low temperatures. It was found that eggs would hatch normal larvae after being kept without water at 10° to 12° C. for forty-two hours.

RESULTS

Egg masses or "sponges" for hatching purposes were detached from the parent by severing each pleopod, to which the eggs are attached, at the base exercising care to keep escaping blood from the eggs. In transporting detached "sponges" to the laboratory, it was found desirable to keep them in jars protected from sunlight and not containing water. "Sponges" were stored at the laboratory awaiting hatching for at least forty-

two hours by maintaining them at temperatures around 11° C. It was found that a very small number of eggs should be placed in the hatching jars to secure best results.

Numerous experiments have shown that, under proper conditions for development, eggs removed from mother crabs hatch and yield vigorous larvae. Eggs requiring as many as fourteen days for development were hatched out successfully in the laboratory. However, in most of the experiments eggs ready to hatch within from one to four days were used. Since the normal incubation period is believed to be about two weeks, it is postulated that eggs may be detached at any time during this period and induced to hatch under laboratory conditions.

Best hatching results were obtained when small numbers of eggs were kept in large containers. Using egg masses that were in good condition, a ninety per cent hatch was readily obtained when the concentration of eggs was about eight per one square centimeter area of bottom surface.

Moulting to the second zoeal stage of this crab is reported for the first time. These zoeae fed on an abundant local dinoflagellate.

Preliminary results of hatching under natural conditions of the York River, in contrast to laboratory conditions, were positive. Although more experiments are still needed, the data seem to indicate definite possibilities for the application of this method as a practical means for producing large numbers of zoeae from sponges that are not now utilized by the industry.

DISCUSSION

The hatching technics described here have direct bearing on how "sponges" now wasted in the crab industry may be utilized for producing larvae. The problem of transporting detached egg masses to the laboratory in a healthy condition has received some attention. When the time required is not over two hours, they may simply be transferred in a jar. However, should large scale handling of "sponges" be undertaken, a need would arise not only for holding them during a longer period required for transportation but also for keeping them in the laboratory during preparation of the necessary conditions for hatching. Storage experiments have shown that eggs kept at a temperature around 11° C. for as long as forty-two hours still hatched on removal to favorable conditions.

While an eighty to ninety per cent hatch has been readily obtained in the laboratory, the findings to date show that a very large hatchery would be needed to accommodate any appreciable daily supply of "sponges". It was for this reason that the York River experiments in natural waters were undertaken. A continuation of these studies is aimed to offer the best results from

an immediate practical standpoint. It is recognized that, although under experimental conditions hatching does take place in the York River waters of twenty parts per thousand concentration, there is no information as to whether or not the larvae are able to survive and moult normally in these waters. That they have moulted into the second zoeal stage in the laboratory suggests the possibility of these waters supporting a normal growth of artificially hatched zoeae, but on this point there is no definite information. More experiments are needed.

Hyman (1920 and 1925) observed that fiddler crabs and some Pinnotherids hatched at dusk, whereas the xanthid, *Menippe mercenaria* seemed to hatch at any hour of day or night. Truitt (1939, p. 15), referring to the hatching of blue crab eggs while they were still attached to the parent, states that hatching takes place during early evening especially about nine o'clock. In our experiments hatching occurred at various hours of day and night and it has not been possible to attach significance to any particular factor as governing the time of emergence of the larvae when, from the observer's standpoint, environmental conditions were favorable.

Having worked out satisfactory hatching technics, it is now possible to obtain an abundant supply of zoeae for studying the environmental conditions that are favorable and unfavorable for the survival of early larval stages. This points the way to an understanding of what natural waters are best suited for hatching and early development.

The development of a relatively easy method for obtaining large numbers of normal, first stage true "zoeae" from detached eggs has additional interest. These zoeae, hatched in the laboratory, serve as a definite basis for the positive separation of blue crab larvae from other closely related larvae that abound in local waters. There is provided the first desirable step in an attempt to obtain successive moults leading through to the "megalops" stage. In experiments conducted during the summer of 1941, first stage zoeae moulted into the second zoeal stage characterized by six setae on each maxilliped. Further shedding failed to take place. It may be pointed out that, for the correct identification of blue crab zoeae in plankton, the importance of observing successive moults from the first true zoeal stage on up to the megalops stage cannot be over-emphasized. Thus in 1941, Dr. S. H. Hopkins at the Yorktown laboratory and Dr. E. P. Churchill working at Hampton and nearby points both report collecting from plankton tows five zoeal stages of *Callinectes sapidus*. Churchill (1941) states "there are a prezoal and five zoeal stages." Hopkins¹ reports that one of the five zoeal stages he has found differs from any of the five found by Churchill, so it would

¹Personal communication.

appear that altogether six zoeal stages occur. Though it is conceded that a large number of random plankton tows might lead to the collection of all the zoeal stages in this particular crab, such success is by no means a certainty since there is, as yet, no proof of the number and identity of all the zoeal stages of this crab. Positive identification of these larval stages from plankton tows is rendered more difficult because of the existence of other Portunids in and near the waters of the lower Bay. (Cowles, 1928, pp. 355-56.)

SUMMARY

Results of laboratory and field experiments on the hatching of detached eggs of the blue crab are presented. There is described—*a*, a technic for removing sponges from the crab and for transporting them to the laboratory from the commercial crab house; *b*, a way of holding sponges for varying periods until facilities are available for hatching; *c*, a technic for removing eggs from the sponge preparatory to hatching; *d*, the technic and environmental conditions that are essential for obtaining a hatching percentage of ninety under laboratory conditions; and *e*, a preliminary experiment on a method of hatching eggs in large numbers in natural waters that offers possibilities for practical application.

Moulting of the first true zoeal stage of the blue crab to the second zoeal stage is reported for the first time.

The application of these findings to a better understanding of the early life history of the crab is defined and the importance of hatching experiments to conservation problems in Chesapeake Bay is defined and discussed.

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