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The effect of environmental factors on hatching, moulting, and survival of zoeal larvae of the blue crab, Callinectes sapidus Rathbun

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THE EFFECT OF ENVIRONMENTAL FACTORS ON HATCHING, MOULTING, AND SURVIVAL OF ZOEA LARVAE OF THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN [1]

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INTRODUCTION

The blue crab constitutes a major fishery of the Chesapeake Bay amounting in 1939 to about fifty-seven million pounds. During the past two decades there have been pronounced fluctuations in the catches attributed to weather conditions and to industrial practices. Thus, from 1939 to 1941 the crab catch declined over 50% in Maryland and about 40% in Virginia (U. S. Fishery Statistics, '41). Since then there has been a marked increase in production.

For successful management of the fishery, further biological information is required to provide a sound basis for conservation policy. The particular biological problem with which we are concerned here is contained in the question, "Where or under what environmental conditions can egg-bearing or "sponge" crabs produce larvae that may be expected to undergo normal development?" An answer to this question provides a basis for selection of crab sanctuaries that may help to protect the brood stock (figure 1). Early students of the blue crab (Churchill, '19) have pointed out the abundance in summer of spawned female crabs near the mouth of the Bay. Some have postulated the possibility of successful spawning and larval development in less saline waters of the Bay at varying distances north of Cape Charles. However, larval distribution records of the Virginia Fisheries Laboratory indicate that blue crab larvae are most abundant in the lower Bay and conspicuously less numerous, during some years at least, in the less saline waters north of the sanctuary located in the general area of Cape Charles (Hopkins, '43). This suggests the importance of salinity as a dominant factor affecting larval development and survival. Since knowledge of the toleration of these larvae to salinity and temperature provides needed information on where and when the crab can propagate most successfully, experiments on how these factors affect hatching and larval development and survival were carried out at Yorktown during the summers of 1941 and 1942. In the course of these studies it has been possible to perfect the technique described by Lochhead and Newcombe ('42) for successfully hatching eggs removed from the crab, to identify beyond question the first three zoeal stages of this crab, to observe the moulting process under different environmental conditions and to provide a sound basis for selecting a crab sanctuary to protect spawning, female crabs during periods of scarcity.

METHODS

Experiments on hatching of eggs and survival of zoeae were carried out by using shallow enamel pans (24 × 20 × 5 cm.) and pint jars. Shallow pans (depth of water, 3 cm.) were used in all experiments not requiring a salinity series under similar temperature. Pint jars proved satisfactory for studying the effect of different salt concentrations on hatching of eggs and on the survival of zoeae (figure 2). In the shallow pan experiments, artificial aeration was not necessary. The water in the pint jars was aerated periodically and the water changed every two days.

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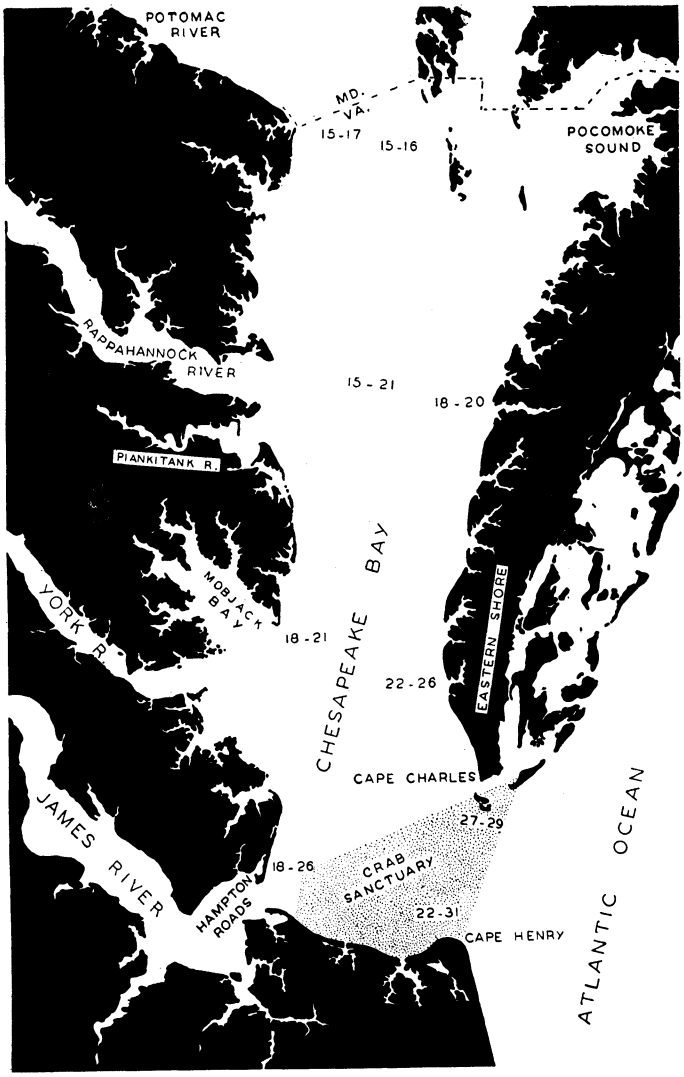

FIG. 1. Showing tidewater Virginia and extent of crab sanctuary (*drawing by G. M. Moore*). Annual average surface-bottom salinity records are indicated (after *Wells, Bailey and Henderson*, 1929).

Some of the egg-bearing crabs were brought to the laboratory alive and the egg strands were clipped from the "sponge" with scissors. In some cases, the egg mass was removed from the crab in the field and placed in an empty jar for taking to the laboratory. The concentration of eggs in the hatching containers was approximately 50 per square centimeter.

For experimental purposes the

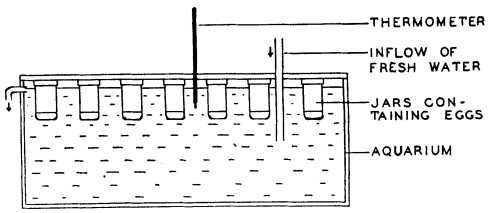

FIG. 2. Showing equipment used in experiments on hatching, moulting and survival of eggs and larvae of the blue crab (*drawn by G. M. Moore*).

"sponges" were designated as—(1) bright orange with yolk completely filling the egg (youngest "sponge," deposition of eggs not completed); (2) pale yellow with yolk filling two-thirds of the egg; (3) dark with brown eye spots well developed, and with body pigmentation; (4) black with eggs about 24–48 hours from hatching, embryos moving within membrane (oldest "sponges").

Different salinities were obtained by slow evaporation of York River water (salinity normally, 17–21 o/oo) and by dilution with distilled water.

Fairly uniform temperatures were obtained by submerging the jars in circulating water and the lower temperatures were obtained in the shallow pans by keeping them in a refrigerator. Temperatures were taken three times daily.

The larvae were observed and fed twice daily. It was found that particular care is required in feeding the zoeae. Daily washing is necessary and miscellaneous food particles must be removed from the container after feeding to prevent the appendages from becoming entangled, a condition which was found to cause high mortality. Best results are obtained by carefully sorting out the desired food from a plankton tow and by removing the excess after feeding.

Data given in tables I–V are based on experiments in which pint jars were used. Shallow pans were used for experiments reported in table VI.

The locations at which eggs were collected and the average water salinities at times of collection, are as follows:

TABLE I. *Showing the average percentage of hatch of blue crab eggs over a salinity range of 0–35 o/oo*

Temperature range between 21.6–22.8° C.

Approximate salinity	Average per cent hatch of 10 experiments
0	0
8	0
9.2	0
10	38
12	49
13	40
14	44
16	43
17	51
Mostly prezoeae up to this point	
18.3	61
21.3	76
25.4	75
27.0	90
28.5	60
30.1	80
32	16
33	0

TABLE II. *Showing effect of salinity on hatching of normal blue crab eggs*

Temperature range: 21.6°–22.8° C. Bright orange "sponge" collected at Pagan's Creek (salinity 17.5 o/oo)—June 25, 1942.

Salinity o/oo	Number of eggs	Per cent hatch
12.8	170	49
13.8	130	55
14.6	139	68
15.8	124	70
18.3	147	61
21.3	144	78
23.5	158	80
26.8	168	85
28.3	142	82

TABLE III. *Showing effect of salinity on hatching of normal blue crab eggs and on the stages of the larvae that emerge*

Temperature range: 25°–29° C. Date of collection—June 16, 1942.

Medium brown "sponge" collected at Sewell's Point (Hampton Roads area)

Salinity o/oo	Number of eggs	Per cent hatch	Remarks
12	190	10	Most prezoeae failed to shed
14.8	160	30	Prezoeae, some 1st. zoeae
18	182	50	Normal zoeae, not very active
20.9	120	70	Normal zoeae
24	189	90	Very active (optimum feeding and activity)
27.8	180	80	Active

Bright orange "sponge" collected from the mouth of the York River

Salinity o/oo	Number of eggs	Per cent hatch	Remarks
14.8	130	10	Prezoeae
17.8	147	30	Prezoeae
20.9	137	50	Normal zoeae
24.6	144	70	Normal zoeae
27.0	162	90	Normal zoeae
30.1	151	80	Normal zoeae

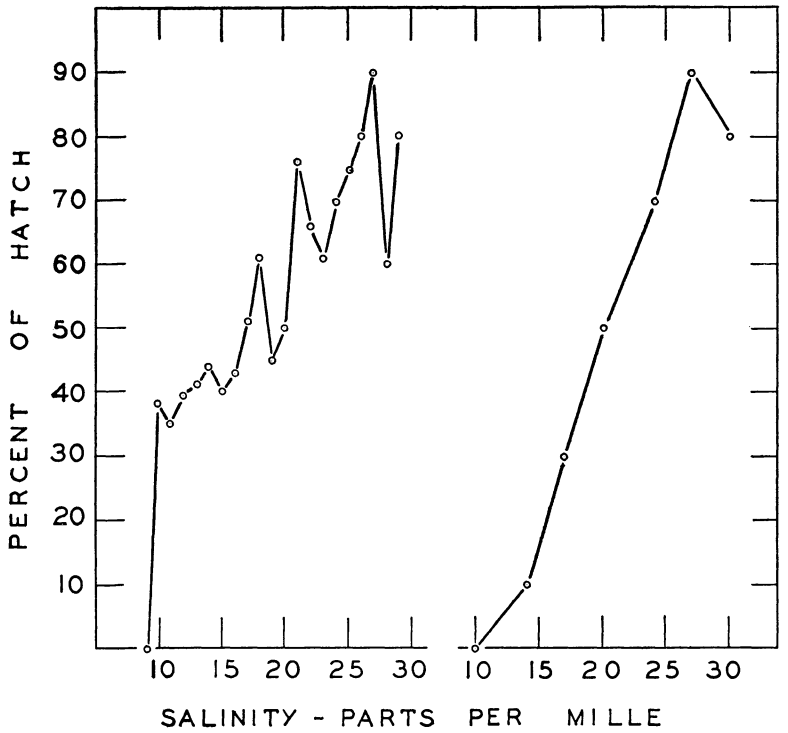

FIG. 3. Showing the relationship of salinity to percentage of hatch of eggs of the blue crab. Left—eggs from Lynnhaven (salinity, 28 o/oo); right—eggs from Pagan's Creek (salinity, 17–18 o/oo).

Lynnhaven, located near Cape Henry, salinity 28.5 o/oo; mouth of the York River, salinity, 20 o/oo; Sewell's Point on the south shore of Hampton Roads, salinity, 21 o/oo; Hampton Bar in Hampton Roads, salinity 19.6 o/oo; Cape Henry, salinity 24.9 o/oo; and Pagan's Creek near the mouth of the James River, salinity 17.5 o/oo.

RESULTS AND DISCUSSION

Hatching. Eggs that were hatched in salinities ranging from 10.3 to 21.3 o/oo required about the same period—namely 9 or 10 days. However, in salinities from 23.5 to 32.6 o/oo eggs hatched from the 11th to the 14th day thus indicating that hatching takes place within a wide physiological limit (figure 3, and table III). Eggs failed to hatch below a salinity of about 9 o/oo or at and above 33 o/oo (table I).

The salinity range for the best hatching results was found to be 23–30 o/oo. Although a few normal zoeae were hatched in salinities as low as 12.8 o/oo, survival was short. In general, the percentage of hatch increased with corresponding salinity increase up to about 23 o/oo (table II). Eggs were hatched successfully at temperatures between 19° C. and 29° C., there being no significant variation in the per cent hatch within this range. At temperatures of 14, 17, 30, and 31° C. all eggs failed to hatch.

The space factor is important in determining a good hatch. Up to about fifty eggs per square centimeter gave satisfactory results.

Normal hatching was considered to have taken place if the larvae at the times of hatching shed the embryonic cuticle (figure 7). The assumption that

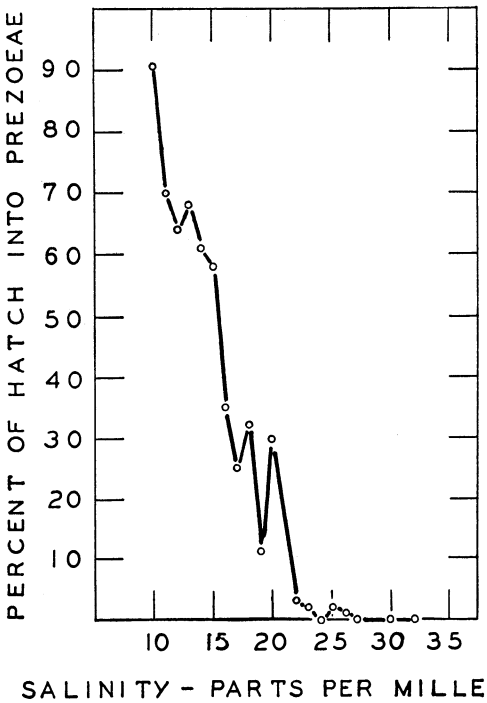

FIG. 4. Showing percentage of hatch of blue crab eggs into prezoeae over salinity range of 0 to 33 o/oo based on average of numerous experiments during period June 16th–Aug. 16th, 1942 (*drawn by G. M. Moore*).

prezoeae do not occur normally in nature if the eggs hatch in waters of optimum salinity is substantiated by these experiments. No prezoeae were obtained in the case of eggs that were hatched under favorable conditions in salinities ranging from 23.4 to 32 o/oo while within the salinity range 10 to 22 o/oo prezoeae did occur. At salinities as low as 10.9 o/oo, 90 to 100 per cent of the larvae remained in the prezoeal stage (figure 4). In all the experiments the percentages of prezoeae increased in proportion to the dilution of the water (table V).

Eggs obtained from "sponge" crabs taken at Hampton always hatched out poorly and showed high bacterial counts (compare tables III and IV).

Churchill ('42) states "the prezoeal stage was not obtained in the towings." In laboratory hatching he concludes "this stage lasted from a half hour to an hour during which time the prezoeae did not swim but remained practically quiet on the substratum. It no doubt does likewise under natural conditions which accounts for the fact that it was not taken in the towing." The experimental data presented in this paper do not support Churchill's statement. They do indicate that unfavorable environmental conditions are responsible for the prezoeal stage during development. These conditions include salt content outside the optimum range and infection by bacteria or fungi (figure 4 and table III).

TABLE IV. *Showing the percentages of hatch of blue crab eggs in different salinities when highly infected by bacteria*

Temperature ranges: 21.6°–22.8° C. Medium brown "sponge" collected at Hampton—June 24, 1942.

Salinity o/oo	Number of eggs	Per cent hatch
15.3	99	16
18	104	9
21.5	115	36
23.7	109	3
27	101	7
29	112	30
31.8	93	16

TABLE V. *Showing the relationship between salinity and number of prezoeae obtained in hatching experiments*

Temperature range: 21.6°–23.8° C.
Eggs collected from the mouth of the York River

Salinity o/oo	Number of eggs	Per cent prezoeae
0	80	0
10	93	91
12	69	63
13	107	69
14	76	61
16	87	35
17	84	25
18.3	68	9
21.3	67	8
25.4	90	0
27.0	93	0
28.5	84	0
30.1	78	0

Eggs collected at Lynnhaven near Cape Henry

Salinity o/oo	Number of eggs	Per cent hatch	Per cent prezoeae
13.3	49	51	20
16.3	65	14	11
19.4	64	75	2
22.4	62	66	0
25.4	73	75	0
28.5	58	60	0

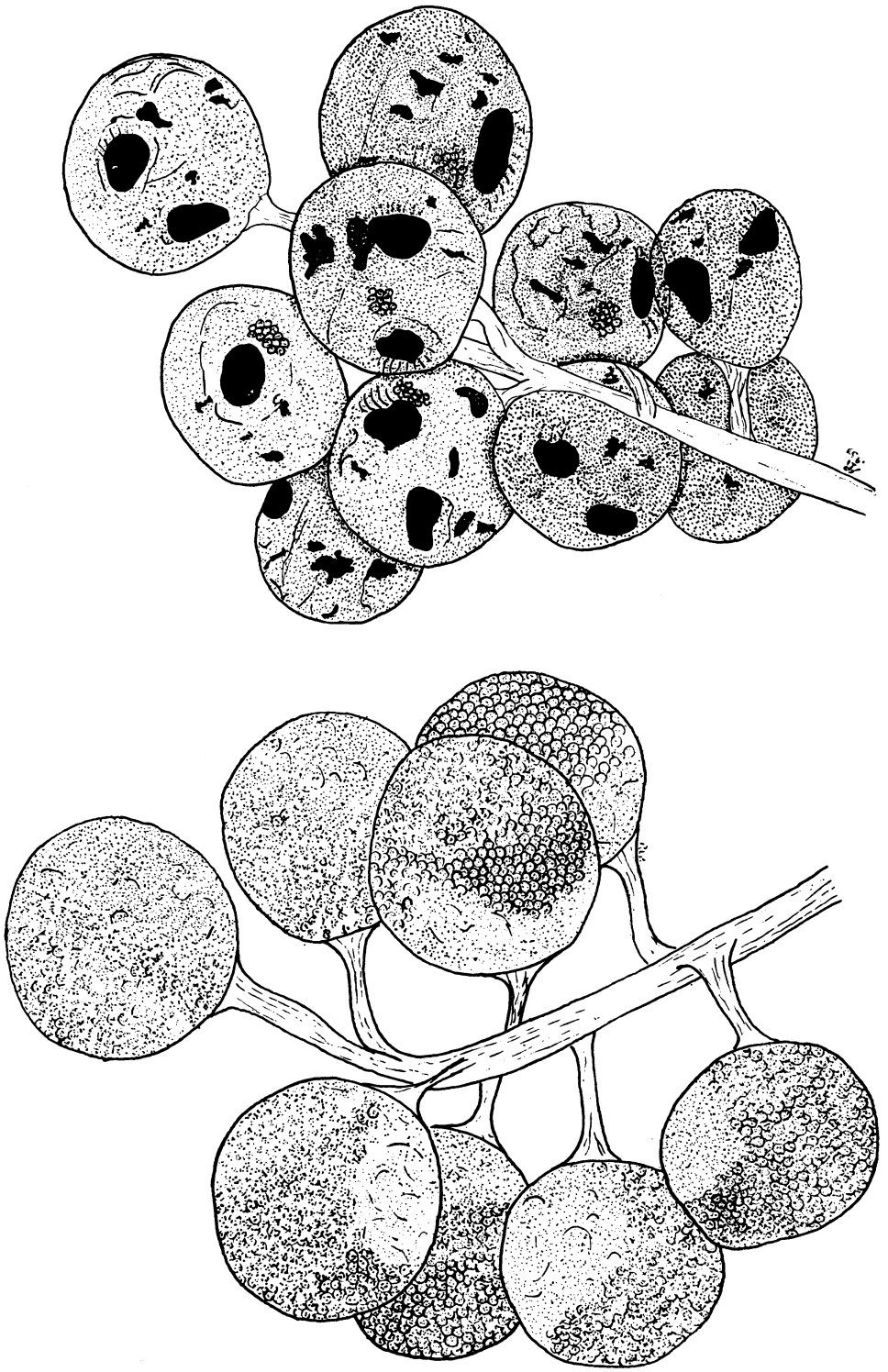

FIG. 5. Left: orange-colored eggs about eleven days before hatching; right: dark-brown eggs shortly before hatching (*drawn by Roy L. Robertson*).

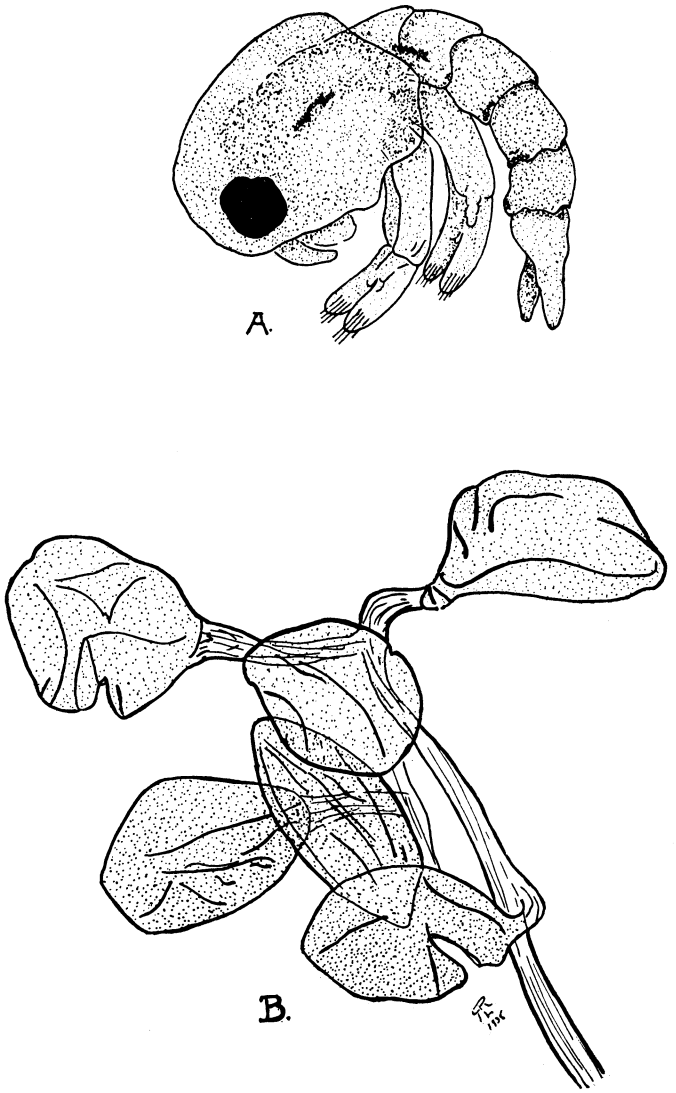

FIG. 6. Above: prezoea larva; below: empty egg shells of the blue crab after emergence of larvae (*drawn by Roy L. Robertson*).

Special attention was given to the emergence of the larvae from the eggs in order to determine whether or not the prezoeae may be considered a normal stage in the development of this species. Under conditions defined here as abnormal, the larva (figure 6A) retains its embryonic cuticle, it lacks the prominent dorsal spine, the telson is undeveloped, and the larva being relatively inactive settles on the bottom. Under normal conditions for development (figure 7) the larva leaves behind the embryonic cuticle and immediately becomes active, having the dorsal spine and a well developed telson. Observations of several thousand prezoeae failed to indicate any that shed from the prezoeae stage into a normal first stage zoeae. Study of the larval stages of mud crabs and fiddler crabs which we have reared through failed to indicate the existence of

prezoeae. Furthermore, Johnson and Lewis ('42) working on the sand crab, *Emerita analoga* (Stimpson), do not report a prezoea. These authors state ". . . the larvae, which quickly become pelagic, hatch in the first zoeal stage."

Survival of Zoeae. In an attempt to determine the salinity requirements of more advanced developmental stages, 24 hours old larvae hatched from the same egg mass were maintained in a salinity series and observed with respect to their behavior and period of survival (table VII). Other environmental conditions such as temperature, light, and food were kept as nearly uniform as possible throughout the series. Salinities below 18 o/oo or above 29 o/oo decreased larval activity. Survival was about the same throughout a temperature range of from 19 to 29° C. Above this temperature the cultures fouled. Bacterial growth was far less in the experiments containing water of lower salinity. Lipman ('26) shows that growth of marine bacteria is materially reduced with dilution of sea water to a salinity of 25 o/oo or below. It was found that introducing water of higher salinity caused an increase in swimming activity and conversely, addition of water of low salinity produced an inactive condition.

A few zoeae obtained from eggs

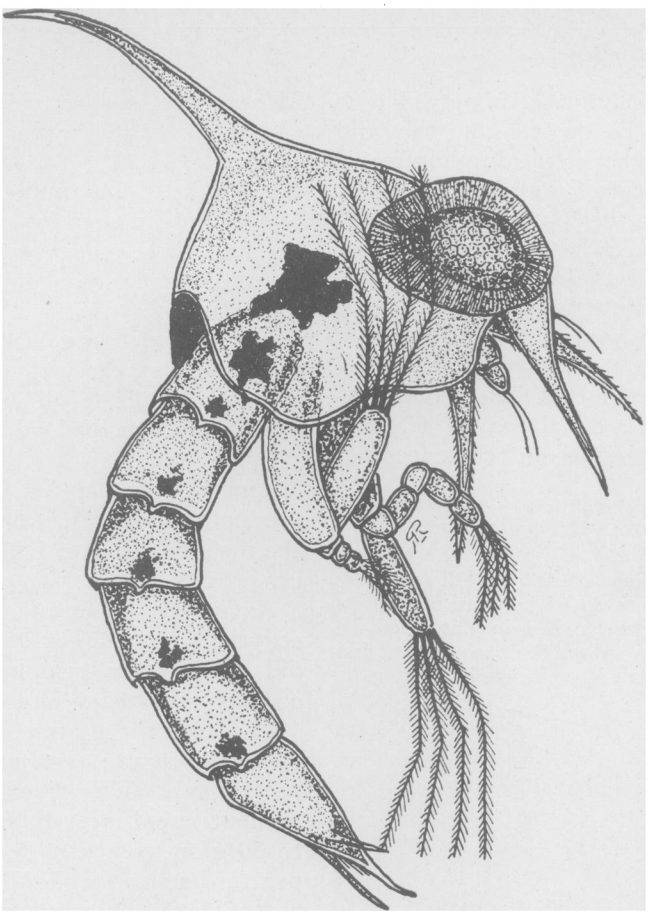

FIG. 7. First zoea larva of the blue crab (*drawn by Roy L. Robertson*).

TABLE VI. *Showing percentage of first zoeae that reached the second zoeal stage on different days following time of hatching*

Percentage for each day in each experiment indicates the total number that reached the second instar.

Experiments	Number of zoeae	Day							Conditions of	
		5th	6th	7th	8th	9th	10th	11th	Salinity o/oo	Temperature °C.
1	75	0	0	0	6	6	10	17.7	26	26–30
2	100	0	0	42	57	64	—	—	26	26–28
3	200	0	0	5	9	35	50	—	25	24–29
4	100	0	34.5	39	45	50	—	—	25	24–26
5	100	0	0	9	9	10	—	—	22–25	24–29
6	70	0	5	22	34	—	—	—	22	24–29
7	75	0	0	0	0	0	0	0	21	24–26*
8	40	0	0	2	6	—	—	—	21–22	25–29
9	100	0	0	0	0	0	0	0	21–22	25–29*
10	100	0	25	52	—	—	—	—	20	24–29
11	200	0	18.5	71	75	—	—	—	20	24–29
12	30	0	0	0	0	0	0	0	23	14–19
13	70	0	0	0	0	0	0	0	21	14–19

* Insufficient food.

hatched at a salinity of about 14 o/oo were found to be sluggish and they did not show the characteristic phototropic responses typical of normal zoeae, such as swarming to the spot of greatest light intensity. When hatched at 18 o/oo, the larvae gave the normal positive reaction to light (active swimming) and at 24 o/oo they were extremely active.

With respect to the temperature factor, it was found that below temperatures of 15° C. or above 29° C. first zoeae become inactive and cease feeding.

TABLE VII. *Showing average time of survival of zoeal stages in a salinity range of 10.6–26.8 o/oo*

Temperature range, 19–29° C. All specimens were zoeae 24 hours old at beginning of each experiment and were hatched in waters of the different salinities indicated. *n* equals not less than 250 individuals in each experiment.

Salinity	Survival in days
10.6	4
11.6	6
13.3	6
15.7	5
16.3	7
17.2	10
18.3	6
20	11
21.3	14
22	16
23.5	20
24.4–26.8	25

Most active feeding took place from 19 to 23° C. when other factors were favorable.

Moulting. Favorable salinity and temperature ranges for ecdysis through the first three stages were found to be from 21 to 28 o/oo and 20 to 29° C. respectively.

The effect of larval concentration on moulting was checked. Isolated zoeae failed to moult in all experiments. It was not determined whether, by their activity, numbers of zoeae stimulated feeding or whether the larvae by some product of their metabolism modified the medium so as to initiate the moulting process.

As to the effect of temperature on ecdysis, at no temperature below 20° C. did a first zoea complete a successful ecdysis. At temperatures from 14 to 19° C. and in the presence of abundant food, the larvae remained in the first zoeal stage. They lived 14 to 20 days without moulting. Salinities from 17 to 30 o/oo were used for the low temperature series (table VI). First stage zoeae passed into second stage zoeae on an average of from 6 to 7 days, and second stage zoeae moulted within 5

to 7 days. The third stage zoeae lived 9 days without passing through another ecdysis. In each case these died in the process of moulting. Poorly nourished individuals moulted only after a relatively long delay.

Contrary to findings on lobsters and similar forms (Templeman, '36), blue crab larvae reared in darkness did not moult, or have as high survival as those reared in north light exposure. In no case were second zoeae obtained from these cultures.

There was a high mortality during the process of moulting from the second to the third zoeal stage although a high percentage of first zoeae reached the second larval stage. The exact percentage of crabs dying in the process of moulting to the third zoeal stage was not determined (table VI). Before moulting, the zoeae showed a tendency to become inactive and refused food. Cast skins were frequently eaten, and larger zoeae often devoured the weaker newly moulted individuals.

In the process of moulting the posterior part of the body is gradually drawn forward and the chitinous cover is slipped off, thus leaving the abdomen and telson free to lash about in aiding the larvae to free the anterior part of the body. This process was observed countless times and varied from a few minutes to as long as two hours for completion.

Food and Feeding of Blue Crab Zoeae. Since feeding is of greatest importance in artificial rearing of blue crab larvae, many types of food were tried. It was found best to remove the larger constituents of the phytoplankton, chiefly diatoms. Zoeae prefer small pelagic organisms especially yellow dinoflagellates (probably *Amphidinium* sp. and *Gymnodinium* sp.). Other foods tried include various unicellular algae; embryos of the oyster drill, *Urosalpinx* and of the oyster, *Ostrea*; small crustacea from plankton tows; detritus containing chloroplasts; the gelatinous material in oyster drill cases; protein particles; yeast; pure culture of *Nitzschia*; small flagellates; egg yolk; minced dried fish; dried clam liver; and small annelid larvae.

No moults were obtained of zoeae that did not receive the yellow dinoflagellates. From plankton tows, it was observed repeatedly during the summer that zoeae and megalops of several species of crabs showed evidence of consuming large numbers of the yellow dinoflagellates.

As soon as newly hatched larvae have used the stored egg yolk they commence to ingest suspended food particles almost continuously. Since the zoeae are transparent, the passage of ingested food through the alimentary canal may readily be observed. The current comes from the dorsal anterior end, the caudal furca pushing particles up into position to be seized by the mandibles. The problem was to introduce very small quantities of the food organism for otherwise the setae of the maxillipeds and mouth parts became hopelessly entangled. After feeding, zoeae were washed with water of the same salinity in which they were kept to free them of extraneous material. Throughout the experiments, contamination of the food medium by the normal physiological processes of the zoeae presented a problem.

In all cases poorly fed (semi-starved) individuals moulted only after a relatively long delay, and in no case were as large as zoeae well fed with the yellow dinoflagellate. Poorly fed larvae required 10 to 13 days to pass from the first to the second instar whereas 5 to 7 days was sufficient for properly fed zoeae. Third zoeae were obtained seven days following increased feeding. These died on the twenty-fifth day without passing into the fourth stage.

The first and second zoeal stages are described by Hopkins ('43). The third stage will be treated in a future publication, Sandoz and Hopkins ('44).

General Remarks. The chief factors that determine successful artificial rear-

ing of blue crab zoeae are proper salinity and temperature, suitable food, and sufficient space for eggs during hatching. Since the structure of a zoea is such that all spiny or filamentous forms cling to it, washing after each feeding plays an important part in the rearing technique. Organisms that readily attach to the zoeae include a stalked vorticellid protozoon, filamentous algae, fungi, diatoms, and numerous internal parasites. When attacked by any or several of these forms in appreciable numbers, zoeae lack vitality and successful moulting does not take place. Mackay ('29) found lobster fry to be parasitized by the diatom, *Licmophora* and by the protozoon, *Acineta*.

Fungus infection of blue crab eggs was first reported during the summer of 1941 when Dr. Margaret Lochhead and the writers observed a high per cent of infection in material collected from Hampton Roads, Virginia (Newcombe '42). In 1942 hatching experiments showed that the fungus infected eggs failed to develop normally (Couch, '42; Sandoz, Rogers and Newcombe, '44).

In laboratory hatching, ecdysis was best between salinities of 21 and 25 o/oo, which correlates with the fact that in plankton tows the greatest concentrations of zoeae in nature have been found in the more saline waters. Moulting took place successfully at temperatures as high as 29° C. and as low as 20° C. Surface temperatures for the Cape Charles–Cape Henry sanctuary area during June, July and August range from about 17 to 27° C. (Cowles, '30).

On a basis of the above experimental data, it is concluded that the salinity and temperature ranges for successful moulting of the early zoeal stages are about 21 to 28 o/oo and 20 to 29° C., respectively. The more advanced developmental stages are increasingly restricted in their ranges of toleration to salinity. While the last zoeal stages were not obtained for study it seems likely that they may be even more restricted in their ranges of toleration than the first three stages.

Turning to the subject of environmental factors operating in the habitat of the egg-bearing crabs, it is known that the summer salinity and temperature ranges near the mouth of the Chesapeake Bay are about 22–28 o/oo and 17–27° C., June to August, inclusive (Cowles, '30). These values compare well with the optimum conditions of early development described for the blue crab.

The area of the lower Bay included in the crab sanctuary established by the Commission of Fisheries of Virginia is indicated in figure 1. That this area constitutes a favorable environment for the development of the early stages of the blue crab is further indicated by the fact that the greatest number of zoeae collected in plankton tows in the Virginia waters of the Bay come from the area of the sanctuary (Newcombe, '42). Numbers as high as 3300 were obtained in a single ten-minute surface tow. At nearby points up the Bay, such as Buckroe Beach and Mobjack Bay, relatively few zoeae were collected, although "sponge" crabs are frequently reported in these waters of lower salt content—17 to 20 o/oo. The experimental results show that there is a correspondence between the optimum toleration points of the zoeae to the salinity factor and the salt content of the waters in the sanctuary. Available evidence, based on a predominance of dark "sponge" crabs in the sanctuary with yellow and orange-colored "sponge" crabs in the less saline waters up the Bay, seems to indicate a migration of "sponge" crabs to the sanctuary preparatory to hatching. Certain commercial fishermen report the presence of crabs with orange and dark-colored "sponges" in low salinity Maryland waters far north of the interstate line but the fate of these eggs is not known.

Our data show that while egg development may proceed at the salinities found in the upper Virginia waters of the

Bay—about 14–18 o/oo, successful moulting of the early zoeal stages should not be expected under these conditions.

Summary

Experimental data are presented that define the approximate conditions for normal development of eggs and early zoeal stages of the blue crab.

Development and hatching of eggs take place under a relatively wide range of salinity and temperature. This range appears to be progressively narrowed in the successive larval instars observed thus far.

The optimum range of salinity for hatching of blue crab eggs is from approximately 23 to 28 o/oo.

Hatching of young, bright, orange-colored eggs under optimum salinity conditions takes place within a period of from 11 to 14 days.

The temperature range, outside of which eggs failed to hatch, is from 19 to 29° C.

Under optimum salinity conditions, prezoeae were not obtained. Reasons are given for believing that the "prezoeal" stage is not a normal one for the blue crab.

The first instar period ranges from six to seven days, and the second from five to seven days under favorable laboratory conditions.

The optimum salinity and temperature ranges for the development of blue crab larvae indicated by laboratory experiments correspond closely with the ranges of these environmental factors that characterize the waters of the Virginia crab sanctuary.

Observation of moulting of the first two zoeal stages of the blue crab is reported, thus establishing the identity of three larval stages of this crab.

Acknowledgments

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