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Blue Crab Larval Culture: Methods and Management

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ABSTRACT–Larval culture methods of the blue crab, Callinectes sapidus, *and its associated food organisms are described in order to provide a guide for rearing larvae for experimental and commercial purposes. Environmental parameters and advantages of controlled rearing of blue crab larvae are discussed. Information on optimal water quality parameters appears to be further developed than that for nutritional requirements of the individual larval stages. Metamorphosis and survival of various blue crab larval stages fed several live food organisms are described with additional discussion of the culture of each food organism.*

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INTRODUCTION

The reasons for annual fluctuations in commercial blue crab landings from the mid Atlantic, south Atlantic, and Gulf Coast areas include: 1) Bad year classes due to low hatchings from the previous year, 2) natural catastrophes, 3) overharvest in previous years, 4) lack of eelgrass or other protective vegetation to provide refuge for soft crabs and smaller juveniles, and 5) pollution of estuarine areas where juveniles feed.

Closed system larval rearing offers an alternative with increased control over environmental parameters. Some advantages of closed system rearing are: 1) No natural catastrophes, 2) no pesticides and other harmful chemicals, 3) no predation, and 4) no cannibalism when individuals are reared separately. The importance of the blue crab to the seafood industry warrants serious consideration of the methods described in this review as a tool of management to supplement fluctuating natural populations. Any experimental work in blue crab larval culture would also benefit from the following discussion.

METHODS

Survival in Natural VS Artificial Systems

The size of a normal egg mass from an ovigerous female has been estimated at $1.75 \times 10^6 - 2 \times 10^6$ eggs by Churchill (1921) and 2×10^6 eggs by Davis (1965) and Warner (1976). Survival to the mature adult under natural conditions has been estimated to be one out of every 2 million eggs produced (Warner, 1976). Under laboratory rearing conditions, Sulkin et al. (1976) reported 22.5-30 percent survival to the megalops stage. Costlow[1] has observed survival, under carefully controlled conditions, to the first crab stage ranging from 0 to 40 percent. The rate of survival of the blue crab increases dramatically under laboratory conditions after reaching the first crab stage. Unpublished results from our laboratory with crabs raised from the first stage to juveniles (ranging from 3 to 7 cm in width) indicated 80 percent survival in a group of 400 individuals maintained in compartmented plastic boxes suspended in glass aquaria containing artificial seawater. Since environmental variables such as temperature, salinity, and dissolved oxygen have been defined for the successful controlled culture of blue crab larvae, a better understanding of their nutritional requirements and feeding behavior would appear to be a major concern in any effort to increase larval survival.

Mating and Spawning

Female blue crabs mate only once in

[1]John D. Costlow, Duke University Marine Laboratory, Pivers Island, Beaufort, NC 28516. Pers. commun.

their lifetime. Mating occurs after their terminal molt in the soft crab stage. Enough sperm is received and stored in the female's spermathecae for two and possibly three spawning periods (Hard, 1942).

Induced mating requires a knowledge of the molt cycle of the male and female. In the mating process, the male should have molted recently while separated from the female, so that the next molt to occur will be that of the female. Otherwise, in the presence of the female the soft crab stage male would be cannibalized. However, the female is protected by the male before, during, and after her terminal molt. Van Engel (1958) describes the coloration of the abdomen of the immature female crab as grayish-white and that of the adult female as blue-green. In the last few days before the final molt of the female, the dark green of the inner, soft, mature abdomen shows through the translucent whiteness of the hard, outer, immature abdomen. This change, in addition to the red line stage on the border of the swimming paddle of the pre-molt female, should provide ample warning that the terminal molt is approaching. Abdominal appearance of the male can also be used to determine its status as a mature or immature individual. According to Van Engel (1958), the abdomen of an immature male is tightly sealed on the ventral surface of the shell, while on a mating male the abdomen hangs free or is held in place by a pair of snap fastener-like tubercles.

Induced mating and spawning under laboratory conditions should include a holding tank with a muddy or gravel bottom to simulate the natural environment. Blue crabs and some other crustaceans such as the freshwater prawn, *Macrobrachium rosenbergii,* appear to abort egg masses frequently on smooth, unnatural bottoms such as glass, fiberglass, and Plexiglass[2]. A minimum of 2-3 months is required after mating before ovulation occurs. In natural waters, such as the Chesapeake Bay, ovarian development may come to a halt during winter months as females cease feeding activity below 10°C (Churchill, 1921). Dredged, overwintering females may be induced to renew ovarian development and subsequent spawning after an acclimation period of increasing water temperatures under laboratory conditions.

Egg Development In Vitro

An alternative to natural egg development and spawning has been provided by Costlow and Bookhout (1960). This method provides several essentials for viable egg development: 1) Adequate aeration, 2) control of fungi and protozoans in the egg mass, and 3) prevention of aborted egg masses which females accomplish through tearing with their walking legs. Eggs are cut from swimmerets previously placed in 30‰ seawater with fine scissors and then further dissociated with glass needles into groups of 100-1,000. The eggs are then placed in plastic compartmented boxes (9 cm^2) containing 20 ml of 30‰ freshly filtered seawater treated with penicillin (200,000 units/l). The boxes are placed on an Eberbach variable speed shaker (110-120 rpm) and maintained at 22-25°C. Hatching of viable eggs was determined at or near 100 percent in compartments containing 100-1,000 eggs. According to Pyle and Cronin (1950), hatching occurs approximately 2 weeks after egg extrusion onto the swimmerets. Churchill (1921) found hatching time, after egg extrusion, to vary slightly with different temperature regimes. At a temperature of 26.1°C (79°F) hatching of eggs required 14-17 days; at 29.4°C (85°F), hatching required 12-15 days. Segregation of limited size egg masses reduced the spread of the fungal egg parasite, *Lagenidium callinectes*. Additionally, by controlling salinity, this method avoids premature larval hatching due to low salinities, which can occur in natural waters (Van Engel, 1958).

The Egg Mass: Age and Disease

According to Bland and Amerson (1974), the color of the egg mass can be used in determining approximate age of the eggs, which can provide a reasonable indication as to how many days are left before a holding apparatus for first stage zoeae should be made available. A yellow to orange color is characteristic of eggs that have been on the swimmerets 1-7 days. Brown to black sponge color indicates 8-15 days have passed since egg extrusion onto the swimmerets.

Two particular diseases of the blue crab egg masses more common than others are: 1) Fungal disease caused by *L. callinectes* and 2) parasitic infestations by the nemertean, *Carcinonemertes carcinophila.* The fungal disease primarily invades immature embryonic stages. Heavily infected eggs can be recognized by their smaller size and greater opacity (Sindermann, 1974). The fungus develops in salinities from 5 to 30‰, spreading rapidly onto the peripheral eggs while avoiding the center of the egg mass. Infected eggs do not hatch and zoeae hatched from eggs previously uninfected may still become infected if left in medium containing fungus spores. Female crabs with infected egg masses should be removed from brood tanks immediately. Immature *C. carcinophila,* living in the gill tissues of the gravid female, migrate to the egg mass after extrusion onto the swimmerets. The worms become sexually mature only in the egg mass. After mating, the female *C. carcinophila* withdraws from its mucus tube, leaving large numbers of eggs within the blue crab egg mass. Development and hatching of nemertean eggs occur within 36 hours. Some migrate back to the gill chambers of the adult female crab, while others swim freely from the host to infest other crabs. However, the damage caused from the nemertean parasite is not as extensive as that caused from the fungal disease.

Characteristics and Optimal Water Quality Parameters for Zoeal Survival and Development

Blue crabs normally go through seven zoeal stages but have survived to the megalops after only six zoeal stages (Sulkin et al., 1976) and even following

[2]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

a supernumerary eighth zoeal stage (Costlow and Bookhout, 1959). Each of the seven normal zoeal stages has morphological changes described in detail by Costlow and Bookhout (1959). Zoeae are heliotropic and free-swimming but are classified as planktonic due to a lack of control of their position in tidal and strong current areas. In natural environments, eggs appear to successfully hatch in salinities of 23-30‰. Costlow and Bookhout (1959) and Sulkin and Epifanio (1975) reported that the optimal temperature for zoeal development is 25°C. Optimal salinity for zoeal development ranges from 26‰ (Costlow and Bookhout, 1959) to 30‰ (Sulkin et al., 1976). Additionally, Sulkin et al. (1976) found synthetic seawater to compare favorably to natural seawater in terms of zoeal survival as 50 larvae (5 sets of 10) in natural water achieved 22.5 percent survival through the megalops stage, whereas a comparable number of larvae in synthetic seawater attained 30.0 percent survival through the megalops stage. Larvae rarely completed their first molt to second stage zoeae in salinities lower than 20‰ or temperatures below 20°C (Costlow and Bookhout, 1959). Mortality of blue crab larvae was highest during the first two zoeal stages (Costlow and Bookhout, 1959). A comparison of environmental parameters used by different investigators is summarized in Table 1.

Total time for zoeal development ranged from 31 to 49 days for the seven stages (Costlow and Bookhout, 1959) compared with an average duration of 35.7 days when only six zoeal stages occurred (Sulkin et al., 1976).

Blue Crab Larval Feeding

Little if any qualitative or quantitative research has been conducted on nutritional requirements of the zoeal stages of the blue crab. With regard to natural foods, several plant and animal organisms have been evaluated for their ability to support survival and growth of larvae to the megalops stage.

According to Costlow and Bookhout (1959), unicellular algae when fed as a sole food source did not provide enough nutrition for successful molting from the first to second zoeal stage, although ingestion by the zoeae was observed for up to 10-13 days, apparently resulting in a somewhat prolonged survival time. Rust and Carlson (1960) observed no apparent utilization of the following phytoplankton by blue crab zoeae: *Gymnodinium, Amphidinium, Chlamydomonas, Platymonas, Isochrysis, Monochrysis, Prorocentrum, Nitzschia, Carteria, Chlorella,* or *Dunaliella*. Possibly one reason that algal species have failed to promote growth of zoeae is that algal diets are totally devoid of animal sterols, which appear essential in the diet of the blue crab. Whitney (1970) found that the blue crab is incapable of de novo synthesis of cholesterol from either acetate-^{14}C or mevalonate-^{14}C. Based on her studies, Whitney theorized that rapid molting and tissue growth in the larval stages require large amounts of sterols for new subcellular membrane formation.

Sulkin (1975) has reared zoeae from the first stage to the megalops with a survival rate of 17 percent on a diet of gastrulae and trochophores from the polychaete, *Hydroides dianthus*. In the same study, rotifer-fed zoeae did not reach the megalops stage, although some survival through the eighth zoeal stage was observed. Mortalities in zoeal stages III, IV, and VII were significantly lower in polychaete-fed crab larvae than in those fed the rotifer diet. Brine shrimp nauplii, *Artemia salina*, alone did not sustain growth from the first to second zoeal stages, and mortality occurred unless either rotifer or sea urchin egg supplements were provided (Sulkin, 1975). Costlow (pers. commun., footnote 1) has also recommended using sea urchin eggs (*Arbacea* sp.) along with freshly hatched *Artemia* nauplii (less than or equal to 12 hours old) for the feeding of zoeal stages I and II. Freshly hatched *Artemia* nauplii may be used as the sole food organism for zoeal stages III-VII. The size of feed organisms appears to determine the blue crab zoea's ability to capture prey (Sulkin and Epifanio, 1975). This would explain the apparent lack of survival of first and second stage blue crab zoeae when brine shrimp nauplii are offered as the sole food.

Table 2 lists the sizes of several live food organisms fed to blue crab zoeae by Sulkin and Epifanio (1975). These investigators concluded that food organisms of 110 μm or less are optimal for the first and second zoeal stages.

Sulkin and Epifanio (1975) made a comparison between groups of stage I and II zoeae fed either sea urchin gastrulae or a rotifer diet. After 14 days, survival in the sea urchin gastrulae-fed group equalled only 5 percent as compared with 50 percent among the rotifer-fed animals.

Sulkin (1975) has suggested that the high degree of success in rearing zoeae through the later stages with brine shrimp nauplii may be due to the high lipid content of the nauplii, which in fact may be required as larval metamorphosis approaches. Sea urchin larvae (derived from isolecithal eggs) are inferior to polychaete and brine shrimp larvae (derived from telolecithal and centrolecithal eggs) in supporting larval metamorphosis of the blue crab to the megalops stage. This may indicate that the type of embryological development determines the nutritional value of the particular food organism as a zoeal food source for the later stage zoeae (Sulkin, 1975). Total lipid per unit dry weight as determined by Sulkin (1975) for several food organisms is as follows: mixed rotifers—8 percent,

Table 1.—Environmental parameters used for zoeal culture.

Item	Costlow and Bookhout (1959)	Sulkin and Epifanio (1975)
Temperature	25°C	25°C
Salinity	26‰	30‰
Photoperiod	12 light/12 dark	14 light/10 dark

Table 2.—Maximum sizes of several live food organisms offered to blue crab zoeae[1].

Organism	Size (μm)
Rotifer (*Brachionus plicatilis*)	45-80
Sea urchin egg (*Arbacea punctulata*)	72-75
Sea urchin egg (*Lytechinus variegatus*)	110
Recently hatched nauplius (*Artemia salina*)	250

[1]From Sulkin and Epifanio (1975).

large rotifers—9 percent, the polychaete, *H. dianthus*—20 percent, and brine shrimp nauplii—30 percent.

Careful selection of potential food organisms, based not only on their ability to promote maximal survival and growth, but also on their freedom from organic and inorganic contaminants as well as biological vectors, is an important consideration for the larval culturist. Bookhout and Costlow (1970) demonstrated the importance of the geographic region from which brine shrimp originate in feeding studies with newly hatched blue crab zoeae fed either San Francisco Bay or Great Salt Lake brine shrimp. Upon analysis for DDT in brine shrimp from the Great Salt Lake, a total of 7,050 parts per billion (ppb) were detected, whereas San Francisco Bay brine shrimp contained approximately 2,300 ppb. Although the length of time required for development to the megalops stage was similar for both brine shrimp-fed groups (about 46 days), there were some indications (Table 3) that the higher pesticide levels detected in Great Salt Lake brine shrimp may have produced greater mortality. Despite the current lack of technical knowledge on the specific nutritional requirements of the blue crab zoeae, with present rearing methods and associated feeding techniques, the larval culturist can be reasonably assured of achieving significantly better survival through the first seven zoeal stages than that known to occur in nature.

The final zoeal stage of the blue crab metamorphoses into the megalops, which precedes the first crab stage or instar of the juvenile period of development. The megalops exhibits benthic as well as planktonic behavior, allowing for better utilization of food throughout the water column than the strictly planktonic zoeal stages. Churchill (1921) reported average sizes of the megalops and the first crab as 0.102 cm and 0.254 cm, respectively. External anatomy of the megalops stage is described in detail by Costlow and Bookhout (1959).

Table 3.—Survival of blue crab larvae fed brine shrimp obtained from two different geographic regions[1].

Region	Percent molted into normal megalops	Percent molted into first crab stage
Zoeae fed *Artemia salina* from San Francisco, Calif.	50	34
Zoeae fed *Artemia salina* from Great Salt Lake, Utah	31	24

[1]From Bookhout and Costlow (1970).

Costlow (1967) fed newly hatched *Artemia* nauplii to blue crab megalops and monitored the effects of various salinity-temperature combinations on survival and rate of development to the first crab stage. The optimal salinity-temperature combination was determined to be 25°C and 30‰ as 100 percent survival was achieved with an average of 8.4 days (6-12 days) required for metamorphosis to the first crab. Sulkin (1975) found that nearly all megalops successfully metamorphosed to the first crab stage when fed *Artemia* nauplii and occasional pieces of fish. However, no megalops reached the first crab stage when fed exclusively on the polychaete, *H. dianthus*. Due to the increased mobility and larger claw size, the megalops of the blue crab is able to prey on *Artemia* nauplii of a larger size range than may be eaten by the zoeae. However, adult *Artemia* should not be fed until the blue crab reaches the first crab stage (Costlow, pers comm., footnote 1).

Holding Tanks for Mass Culture of Blue Crab Larvae

A combination larval hatching-collection chamber designed by Smith and Hopkins (1977) for separating newly hatched freshwater prawn, *M. rosenbergii*, from egg-bearing females appears suitable for use with the blue crab (Fig. 1). This system utilizes air lift pumps that discharge 7.6 liters/minute of water into the hatching chamber, providing a current which carries larvae through a nylon screen (0.6 cm mesh size) into the collection chamber. Light above the collection chamber causes positive phototactic response of crustacean larvae to concentrate most individuals near the surface for easy retrieval. Because outstretched stage I blue crab zoeae range from 300 to 400 μm and sphere-shaped zoeae range from 200 to 300 μm, a screen with a 100 μm mesh size is recommended for separation of the larval collection chamber from the biological filter section to prohibit flow of larvae past the larval collection chamber. As a result of using a smaller screen mesh size than that recommended for freshwater prawn, differences in head loss may be reduced through decreased flow rate or increase in surface area of the screen.

Serfling et al. (1974) designed a recirculating culture system (Figs. 2, 3) for larvae of the American lobster, *Homarus americanus*, which is a modified version of an earlier system developed by Hughes et al. (1974). It would appear that Serfling's sytstem offers a practical holding system approach for the mass rearing of blue crab larvae even though the first two zoeal stages would have to be separated from the last five zoeal stages due to different food organisms being offered (i.e., rotifers for zoea I and II and brine shrimp nauplii for zoea III to VII). Either a duplicate system or compartmented boxes would be required for the first two zoeal stages. The system described by Serfling et al. (1974) has a 10 liter/minute flow rate for larval dispersal and provides primary filtration through a graded sand (No. 12 silica) and crushed oyster shell filter bed. Secondary filtration includes a charcoal and fiber filter used to remove chemical contamination and residual particulate wastes. Serfling et al. (1974) also discusses the use of heating elements as well as occasional ultraviolet sterilization of the water, together with antibiotics.

Culture of Feed Organisms for Blue Crab Larvae

Environmental parameters, algal species, and feeding rates, which provide optimal survival and development for the culture of the rotifer, *Brachionus plicatilis*, and the brine shrimp, *A. salina*, will be described in order to provide ample food for blue crab larvae in a larval culture operation.

The rotifer, *B. plicatilis*, is described as a mixohaline species because of its

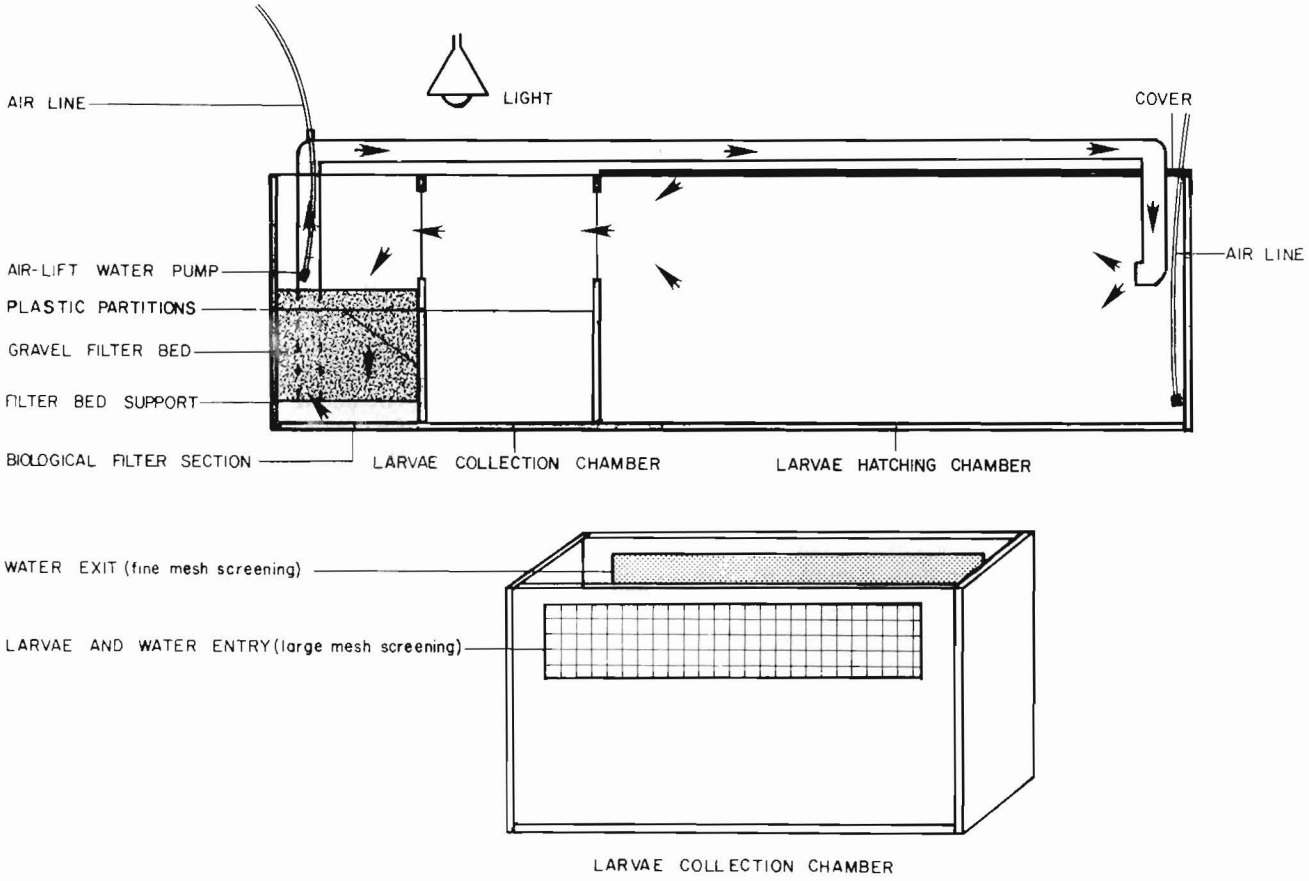

Figure 1.—Longitudinal and frontal view of hatching-collection chamber (Smith and Hopkins, 1977).

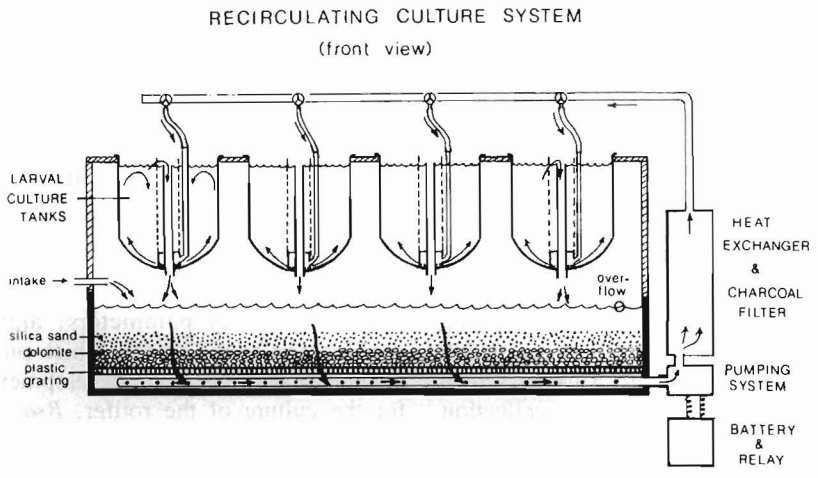

Figure 2.—Front, cross-sectional view of the recirculating culture system, showing the Hughes Culture Tank and the Circulator design and the primary and secondary filtration systems. Illustration is from Serfling et al. (1974), courtesy of Elsevier Scientific Publishing Company, Amsterdam, The Netherlands.

tolerance of a wide salinity range (0.5-40‰) without any apparent adverse effects (Theilacker and McMaster, 1971). This species is easily obtained and cultured as a result of its wide tolerance to variable environmental parameters. To maintain an optimal environment for both rotifer culture and its algal food source, a water salinity of 25‰ and a temperature of 21°-25°C were established by Theilacker and McMaster (1971). These conditions were based on observations that the reproductive rate of *B. plicatilis* was similar in either 25 or 30‰ seawater and the fact that growth of the algal food source, *Dunaliella* sp., appeared optimal at a salinity of 25‰. Based on experimentation with four algal genera, *Dunaliella, Nannochloris, Exuviella,* and *Monochrysis,* Theilacker and McMaster (1971) concluded that the

unicellular flagellate, *Dunaliella* sp., was the most practical choice. This species did not require vitamins or soil extract in the culture medium. For culture of *Dunaliella,* ultraviolet-treated 24‰ seawater was filtered through two Cuno Aqua-pure filters (pore size=5 μm), a large capacity millipore cartridge pre-filter and filter (pore size=0.45 μm), and reirradiated with ultraviolet light. Sixteen liters of medium maintained at a temperature of 24°±1°C in 20-liter carboys were bubbled with 5 percent carbon dioxide and agitated with a magnetic stirrer under constant illumination at a light intensity of between 500 and 700 footcandles. Cell numbers were found to increase from 3.4×10^3 to 3×10^5 cells/ml in 9 days. At peak growth, 25 percent of the algal culture is removed daily and replaced with fresh media for 10 additional days. For mass rotifer production, fiberglass tanks were filled to a depth of 13 cm (464 liter volume) and inoculated with 32 liters of *Dunaliella* culture to yield a final concentration 2×10^5 cells/ml. When *Dunaliella* concentrations reached 1×10^6 cells/ml (2-4 days), the fiberglass tanks were charged with approximately 2×10^4 rotifers. The tanks were then aerated under constant illumination at an intensity of 140-170 footcandles. This procedure supported a production of 2.5×10^6 rotifers/day within a 4 or 5 day period. The size of *B. plicatilis* ranged from 99 to 281 μm. Average rotifer weight equalled 0.16 μg with an energy content of an individual animal determined to be 8×10^{-4} calories. An average caloric content was estimated at 5,335±139 cal/g of ash-free dry weight. Rotifer concentrations as high as 200/ml did not inhibit reproduction. Separation of rotifers from the algal materials was accomplished with the aid of a submersible pump and plastic tube covered at one end with 64 μm mesh netting (Theilacker and McMaster, 1971).

Techniques for producing newly hatched brine shrimp nauplii have been thoroughly described by Sorgeloos and Persoone (1975). Hatching of cysts in seawater was found to require a salinity between 5 and 70‰. However, Boulton and Huggins (1977) compared hatching rates in salinities of approximately 0, 17.5, 35, 52.5, and 70‰ seawater after hydration for 6 hours as well as after subsequent 3-hour intervals up to 30 hours. No free swimming nauplii were observed in 50 percent diluted seawater after 30 hours of hydration. Highest percentage hatching occurred in full-strength seawater after 27 hours (63±11 percent) and 30 hours (69±9 percent). Hatching rate in higher salinities was greater with each additional 3-hour interval from initial hydration but remained lower than that achieved in full-strength seawater. For "Californian cysts," illumination for 10 minutes at an intensity of approximately 185 footcandles (2,000 lux) in a medium containing at least 3 ppm dissolved oxygen is sufficient for successful hatching (Sorgeloos and Persoone, 1975). These same authors have reported an optimal temperature to equal approximatley 30°C. Foaming (protein bubble formation), which is caused by decomposed byproducts of dead *Artemia,* can be minimized by using a few drops of nontoxic silicone antifoamer. A cylindrical separator box constructed by Sorgeloos and Persoone (1975) capitalizes on the positive phototactic behavior of newly hatched nauplii and is an improvement over rectangular bottom containers used in the past. This separator avoids accumulation of cysts and newly hatched nauplii in corners, which causes low hatching and poor survival rates.

Culture of brine shrimp beyond the newly hatched stage is recommended in order to feed the megalops stage and early instars of the juvenile period. The phytoflagellate, *Dunaliella viridis,* is a satisfactory feed organism for newly hatched nauplii. Funnel-shaped bottoms for algal culture vessels (Fig. 4, 5) eliminate sedimentation and permit circulation of nutrients (Persoone and Sorgeloos, 1975). Some investigators have used a supplemental source of CO_2 for algal culture, whereas others,

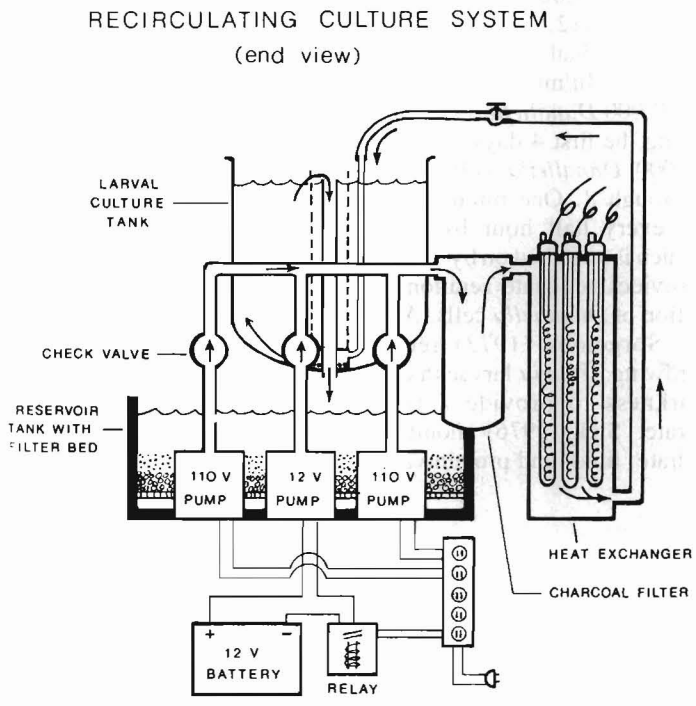

Figure 3.—End, cross-sectional view of the recirculating culture system, showing the secondary filtration and back-up power systems. Illustration is from Serfling et al. (1974), courtesy of Elsevier Scientific Publishing Company, Amsterdam, The Netherlands.

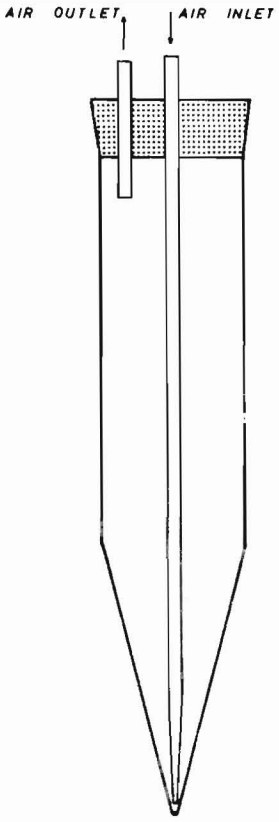

Figure 4.—Algal culturing tube, 100 ml (Persoone and Sorgeloos, 1975).

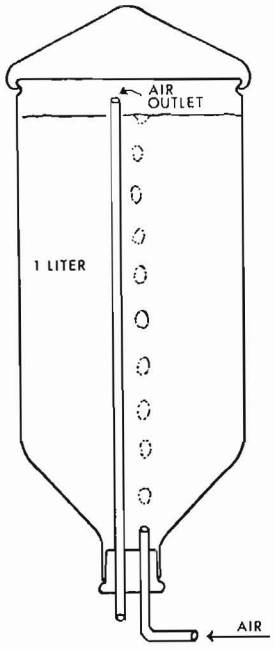

Figure 5.—Glass serum bottle used for culturing algae. (Persoone and Sorgeloos, 1975).

such as Persoone and Sorgeloos (1975), insist that in high density cultures, optical density of the culture is more limiting than insufficient amounts of CO_2. The use of axenic cultures for algal production is not generally encouraged due to: 1) Low amount of bacteria present during the exponential growth phase of the algal colony, and 2) the high expense and cumbersome work involved in keeping a bacteria-free culture. Persoone and Sorgeloos (1975) suggested the following stock solution for culture of *Dunaliella* sp.:

$FeSO_4 \cdot 7H_2O$	0.278	g
$NaH_2PO_4 \cdot 2H_2O$	3.0	g
$NaNO_3$	30.0	g
$MnCl_2 \cdot 4H_2O$	0.47	g
Glycocol	50.0	g
Distilled water	1	liter

In high density culture experiments with brine shrimp nauplii, Sorgeloos (1973) achieved concentrations of between 1 and 3 nauplii/ml at a temperature of 28°C as follows: 1) 2,000 individuals in 1-liter containers (2 nauplii/ml); 2) 25,000 individuals in 10-liter containers (2.5 nauplii/ml); and 3) 50,000 individuals in 30-liter containers (1.6 nauplii/ml). Each nauplius required 50,000 *Dunaliella* cells twice daily during the first 4 days of culture and 100,000 *Dunaliella* cells during days 5 through 8. One minute of air bubbling every half hour by an air pump, which is switched on by a timing clock, provides adequate aeration and recirculation of *Dunaliella* cells. Additionally, Sorgeloos (1973) recommends growing *Artemia* larvae in complete darkness to provide a faster growth rate. Sick (1976) monitored carbohydrate, lipid, and protein values for several phytoplankton species fed to brine shrimp larvae and found better growth in larvae fed high protein-lipid species, such as *D. viridis* and *Chlamydomonas sphagnicolo,* as opposed to high carbohydrate species, such as *Nitzschia closterium*. Paffenhöfer (1967) determined the average content of newly hatched *Artemia* nauplii to be 5,953 cal/g ash-free dry weight.

Use of dry powder forms of a feed organism as a substitute for live cultures minimizes the number of living links necessary to culture the desired species. Person - LeRuyet (1976) has developed a technique for intensive rearing of brine shrimp larvae using the blue-green alga, *Spirulina maxima,* in dry powder form. On a weekly basis, productions of 75 g of dry matter of brine shrimp from a 450-liter tank were attained. Table 4 summarizes optimal concentrations of larvae and the amount of dry powder required. The average size of an individual larva as well as optimal concentration compares favorably with previous results attained by Sorgeloos (1973) using live food for *Artemia* larvae.

SUMMARY

Extensive research by many investigators has provided useful information on the culture of feed organisms for use in blue crab larval culture. Each food organism has its own environmental parameters for optimal survival and growth. Often, this dictates a compromise of environmental parameters between any two adjacent organisms in the food chain, e.g., *Dunaliella* and *Artemia* or *Artemia* and *Callinectes*. As more knowledge of nutritional requirements is gained, formulated feeds could become practical for economic

Table 4.—Growth of larval *Artemia salina* as influenced by algal food concentration and larval density[1].

Days	No. of larvae	Amt. (mg) of powder[2] offered	Brine shrimp larva size $\bar{X}$ (mm)	Adjusted larval concentration/ml
0-2	10,000	600	1 (after 2 days)	13-14
3-4	10,000	1,800	2 (after 4 days)	5
5-6	10,000	4,300	3.75 (after 6 days)	2

[1]From Person - LeRuyet (1976).
[2]Blue-green alga, *Spirulina maxima,* in dry powder form.

purposes as well as easing the amount of work associated in culturing food organisms required for blue crab larvae.

Presently, it appears that brine shrimp nauplii, in combination with sea urchin eggs or rotifers, may be fed successfully to the first two zoeal stages, while brine shrimp nauplii appear to be the best available food organism for the subsequent zoeal stages (III-VII). The megalops stage requires brine shrimp nauplii in order to metamorphose into the first crab stage under laboratory conditions.

Despite extensive work by several investigators, survival of blue crab larvae under laboratory conditions remains below that attainable with other crabs (i.e., the mud crab, *Rhithropanopeus harrisi,* and the stone crab, *Menippe mercenaria*). Although highly variable, Costlow (pers. commun., footnote 1) has indicated a maximum survival rate to the first crab stage of approximately 40 percent.

Studies monitoring the nutritional requirements of juvenile crabs have been highly successful when using hatchery-raised individuals as opposed to individuals captured from natural waters (Biddle, pers. commun., footnote 3). Juveniles obtained from natural waters frequently succumbed to disease after transfer to laboratory holding systems, whereas hatchery-raised crabs did not exhibit similar problems.

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LITERATURE CITED

Bland, C. E., and H. V. Amerson. 1974. Occurrence and distribution in North Carolina waters of *Lagenidium callinectes* Couch, a fungal parasite of blue crab ova. Chesapeake Sci. 15:232-235.

Bookhout, C. G., and J. D. Costlow, Jr. 1970. Nutritional effects of *Artemia* from different locations on larval development of crabs. Helgol. Wiss. Meeresuters 20:435-442.

Boulton, A. P., and A. K. Huggins. 1977. Biochemical changes occurring during morphogenesis of the brine shrimp *Artemia salina* and the effect of alterations in salinity. Comp. Biochem. Physiol. 57A:17-22.

Churchill, E. P., Jr. 1921. Life history of the blue crab. Bull. U.S. Bur. Fish. 36:95-128.

Costlow, J. D., Jr. 1967. The effect of salinity and temperature on survival and metamorphosis of megalops of the blue crab *Callinectes sapidus*. Helgol. Wiss. Meeresunters 15:84-97.

———— and C. G. Bookhout. 1959. The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. Biol. Bull. (Woods Hole) 116:373-396.

———— and ————. 1960. A method for developing Brachyuran eggs *in vitro*. Limnol. Oceanogr. 5:212-215.

Davis, C. C. 1965. A study of the hatching process in aquatic invertebrates: XX. The blue crab, *Callinectes sapidus*, Rathbun, XXI. The nemertean, *Carcinonemertes carcinophila* (Kölliker). Chesapeake Sci. 6:201-208.

Hard, W. L. 1942. Ovarian growth and ovulation in the mature blue crab, *Callinectes sapidus* Rathbun. Chesapeake Biol. Lab., Publ. 46, 17 p.

Hughes, J. T., R. A. Shleser, and G. Tchobanoglous. 1974. A rearing tank for lobster larvae and other aquatic species. Prog. Fish-Cult. 36:129-133.

Paffenhöfer, G.-A. 1967. Caloric content of larvae of the brine shrimp *Artemia salina*. Helgol. Wiss. Meeresunters 16:130-135.

Person - LeRuyet, J. 1976. Rearing of *Artemia salina* (Branchiopoda) larvae on inert food: *Spirulina maxima* (Cyanophycea) (In French, Engl. summ.) Aquaculture 8:157-167.

Persoone, G., and P. Sorgeloos. 1975. Technological improvements for the cultivation of invertebrates as food for fishes and crustaceans. I. Devices and methods. Aquaculture 6:275-289.

Pyle, R., and E. Cronin. 1950. The general anatomy of the blue crab, *Callinectes sapidus* Rathbun. Chesapeake Biol. Lab., Publ. 87, 40 p.

Rust, J. D., and F. Carlson. 1960. Some observations on rearing blue crab larvae. Chesapeake Sci. 1:196-197.

Serfling, S. A., J. C. Van Olst, and R. F. Ford. 1974. A recirculating culture system for larvae of the American lobster, *Homarus americanus*. Aquaculture 3:303-309.

Sick, L. V. 1976. Nutritional effect of five species of marine algae on the growth, development, and survival of the brine shrimp *Artemia salina*. Mar. Biol. (Berl.) 35:69-78.

Sindermann, C. J. 1974. Diagnosis and control of mariculture diseases in the United States. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Middle Atl. Coastal Fish. Cent., Highlands, N.J., Tech. Ser. Rep. 2, 306 p.

Smith, T. I. J., and J. S. Hopkins. 1977. Tank designed for hatching and collecting larvae of the giant Malaysian prawn, *Macrobrachium rosenbergii*. Prog. Fish-Cult. 39:182-183.

Sorgeloos, P. 1973. High density culturing of the brine shrimp, *Artemia salina* L. Aquaculture 1:385-391.

————and G. Persoone. 1975. Technological improvements for the cultivation of invertebrates as food for fishes and crustaceans. II. Hatching and culturing of the brine shrimp, *Artemia salina* L. Aquaculture 6:303-317.

Sulkin, S. D. 1975. The significance of diet in the growth and development of larvae of the blue crab, *Callinectes sapidus* Rathbun, under laboratory conditions. J. Exp. Mar. Biol. Ecol. 20:119-135.

————, E. S. Branscomb, and R. E. Miller. 1976. Induced winter spawning and culture of larvae of the blue crab, *Callinectes sapidus* Rathbun. Aquaculture 8:103-113.

————and C. E. Epifanio. 1975. Comparison of rotifers and other diets for rearing early larvae of the blue crab, *Callinectes sapidus* Rathbun. Estuarine Coastal Mar. Sci. 3:109-113.

Theilacker, G. H., and M. F. McMaster. 1971. Mass culture of the rotifer, *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar. Biol. (Berl.) 10:183-188.

Van Engel, W. A. 1958. The blue crab and its fishery in Chesapeake Bay Part 1. - Reproduction, early development, growth, and migration. Commer. Fish. Rev. 20(6):6-17.

Warner, W. W. 1976. Beautiful swimmers. Little Brown & Co., Boston, 304 p.

Whitney, J. O'C. 1970. Absence of sterol biosynthesis in the blue crab *Callinectes sapidus* Rathbun and in the barnacle *Balanus nubilus* Darwin. J. Exp. Mar. Biol. Ecol. 4:229-237.

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