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The development of blue crabs, Callinectes sapidus, from kepone-contaminated eggs

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THE DEVELOPMENT OF BLUE CRABS, *CALLINECTES SAPIDUS*, FROM KEPONE-CONTAMINATED EGGS

A THESIS

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Master of Arts

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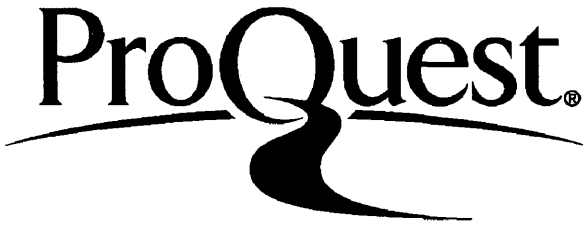
by

A. Thomas Leggett, Jr.

1979



APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts in Marine Science

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DEDICATION

This thesis is dedicated to Pappy and Moma Ruth, my grandparents, and to my father and mother. It was their constant guidance and love that inspired in me the desire to study marine science.

ACKNOWLEDGEMENTS

Many thanks go to my major professor, Dr. Morris H. Roberts, Jr. for his guidance and help throughout my degree program. My appreciation is also extended to my other thesis committee members for their help during my thesis research.

Field collections were made by Jimmy Greene, Bob Bendl, and John Reynolds. Bob Bendl, John Reynolds, Morris Roberts, Jerry Illowsky, and Mary B. Soles assisted in laboratory dissections of adult blue crabs and culture of eggs and larvae. Harold Slone and his colleagues of the Department of Ecology Pollution, of the Virginia Institute of Marine Science, performed all of the Kepone analyses. This research was funded by the Virginia State Kepone Fund and in part by the Virginia Institute of Marine Science.

My wonderful wife Kimberly deserves a great deal of credit for tolerating a seemingly endless research project. She also excused me, on a temporary basis, from many of my household chores while I prepared my thesis.

My other family members, in-laws included, were also most patient during my research endeavors.

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ABSTRACT

Ovigerous female blue crabs were collected from several areas in the lower Chesapeake Bay and lower James River over a three month period during the summer 1978. In addition ovigerous crabs were collected from two seaside stations in August of the same year. Hatchability of several hundred eggs from each crab was determined. An aliquot of larvae from each female was cultured to determine survivorship to the first crab. The remainder of the egg mass was analyzed for its Kepone content. Levels of Kepone up to 1.45 μg/g in eggs appeared to have no effect on any aspects of embryogenesis, hatchability, or larval survival.

THE DEVELOPMENT OF BLUE CRABS, *CALLINECTES SAPIDUS*, FROM KEPONE-CONTAMINATED EGGS

INTRODUCTON

The introduction of Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalene 2-one) into the James River at Hopewell, Virginia, has caused widespread concern for the major fisheries of the Chesapeake Bay. Kepone, developed and produced by Allied Chemical Corporation and Life Science Products of Hopewell, was used to control ants, cockroaches, and other insect pests of potatoes and bananas (Anonymous, 1978). Documented environmental contamination by Kepone only dates back to 1974 (Anonymous, 1978). A detailed account of the chronology of Kepone contamination, the implications of pollution by the pesticide, and future mitigation plans can be found elsewhere (Anonymous, 1978).

Studies to determine the sinks and routes of transport for Kepone in the James River showed that the pesticide has a high affinity for fine organically rich sediment particles (Trotman and Nichols, 1978). The main sink for Kepone is the James River bed sediments rather than the water column. Kepone concentrations of 150 ppb and greater were common for sediments in the area of the James River turbidity maximum whereas those for sediments of Hampton Roads and the Chesapeake Bay were approximately 10 ppb. The major transport routes for Kepone involve biological mechanisms such as accumulation directly from solution and through various trophic levels from sediments or food materials.

Only a limited number of Kepone uptake and depuration studies have been conducted. Walsh et al. (1977) have shown that phytoplankton are capable of concentrating Kepone from water which is then available to higher trophic levels. Bivalves (Crassostrea virginica, Macoma balthica, and Rangia cuneata) can concentrate Kepone from contaminated suspended sediments by factors thousands of times greater than that in suspended sediments (Haven and Morales-Alamo, 1977). Alosid fish, such as alewife, shad, and herring, filter water to extract small particles of food. Kepone may enter the fish either by diffusion through the gill membranes or by ingestion of Kepone-laden particulate matter (Anonymous, 1978). Top carnivores such as bluefish which enter the James River virtually Kepone-free, may accumulate Kepone above the acceptable Action Level (maximum permissible concentration allowable in food sold to the public, set by the U.S. FDA; 0.3 μg/g for fish) in a matter of weeks (Bender et al., 1977).

Blue crabs from the James River contained 0.81 μg/g Kepone in males and 0.19 μg/g in females. The difference was attributed to males spending more time in river systems than females (Bender et al., 1977). Roberts (1980) also found that male blue crabs contained much more Kepone in the back fin muscle than females. Female crabs concentrated Kepone in the gonad, and, to a lesser extent, the hepatopancreas, whereas males did not. Egg masses from ovigerous females contained more Kepone than did ovaries. Ovigerous females, especially those with spent ovaries, contained more Kepone in backfin

muscle than non-ovigerous females. Roberts and Leggett (1980) have shown egg extrusion to be a significant route for Kepone depuration in female blue crabs.

Several toxicity studies have been conducted to determine the degree to which Kepone affects estuarine organisms. Kepone was acutely toxic to lugworms (Arenicola cristata) at a concentration of 29.5 µg/l (Rubenstein, 1977). The 96-hour LC50 for the estuarine mysid, Mysidopsis bahia, exposed to Kepone in water was 10.1 µg/l (Nimmo et al., 1977). The 19-day LC50 for M. bahia was 1.4 µg/l. The 96-hour LC50 value for grass shrimp, Palaemonetes pugio, was 121 µg/l, for the blue crab, Callinectes sapidus, greater than 210 µg/l, for the sheepshead minnow, Cyprinodon variegatus, 69.5 µg/l, and for spot, Leistomus xanthurus, 6.6 µg/l (Schimmel and Wilson, 1977). At the concentrations tested, Kepone was acutely toxic to all organisms except blue crabs.

The blue crab is one of the most important Chesapeake Bay fishery resources. This species has been fished intensively for over 100 years with annual Bay landings usually exceeding one million pounds. Churchill (1919) and Van Engel (1958) give extensive reviews of the blue crab fishery and life history. Mating begins in early May and continues through fall. Females store sperm in seminal receptacles, allowing two or more spawnings without further copulation. After mating in river waters, females migrate to higher salinity waters at the Chesapeake Bay mouth for overwintering. Males remain buried in river muds. Spawning occurs several months after mating, mainly

between May and September. Eggs are carried as a large "sponge" on the female abdomen for approximately two weeks prior to hatching. A sponge may contain 700,000 to 2,000,000 eggs. Larval duration is usually 55 to 60 days in the laboratory. Larval food, based on laboratory studies, is thought to consist of zooplankton, while juveniles and adult crabs eat fresh or decaying organisms including bivalves (Menzel and Nichy, 1958; Van Engel, 1958).

The extent to which Kepone pollution in the Chesapeake Bay has affected the blue crab fishery is questionable. Schimmel et al. (1979) observed that decreases in Chesapeake Bay blue crab landings coincided with Kepone contamination in the James River. Since the strength of a fishery is measured from reported landings, closure of the James River to blue crab fishing complicates determination of the impact of Kepone on the James River stock.

A limited number of reports deal with Kepone toxicity to blue crabs. Schimmel and Wilson (1977) showed Kepone dissolved in sea water to be nontoxic to adult blue crabs at 210 µg/l. Butler (1963) reported 20% blue crab mortality at 1.0 mg/l after 48 hours. The crabs were only "irritated" after 24 hours at the same concentration. Schimmel et al. (1979) reported uptake and depuration of Kepone in blue crabs fed Kepone-contaminated oysters, Crassostrea virginica. Crabs fed James River oysters containing 0.15 µg/g Kepone exhibited greater mortality and molted fewer times than crabs fed Kepone-free oysters. Crabs fed oysters containing 0.15 µg/l Kepone (produced by

laboratory exposure) molted fewer times than crabs fed control oysters but mortality was not significantly different from control crabs.

When comparing bioassays in which Kepone was dissolved in water with those in which Kepone was contained in food, it becomes obvious that doses in water, two orders of magnitude greater than those in food materials, do not cause crab mortality (e.g., compare Schimmel and Wilson, 1977; Schimmel et al., 1979). This can be attributed to the fact that Kepone does not readily pass from water to the crab in significant quantities. This phenomenon has also been demonstrated in studies of Mirex and DDT toxicity to blue crabs (Butler, 1963; Leffler, 1975; Lowe et al., 1977; Mahood et al., 1970).

Only one study has been reported which deals with the effects of Kepone on crab larvae (Bookhout et al., 1979). However, several papers have been published concerning the effects of other pesticides on decapod larvae (Bookhout and Costlow, 1975; Bookhout and Monroe, 1977; Bookhout et al., 1976; Bookhout et al., 1972, Buchanan et al., 1970; Epifanio, 1971; Fisher et al., 1976)

Bookhout et al. (1979) reported decreased survival and molting rate of blue crab larvae with increased concentrations of Kepone.

Using the closely related compound Mirex, Bookhout and Costlow (1975) reported that during the first five days of exposure, concentrations of Mirex up to 10 μg/g produced no effect on blue crab larvae. After five days the higher concentrations reduced survival. Residue analysis of larvae indicated that larvae reared in the higher

concentrations of Mirex contained more Mirex than larvae reared in lower concentrations of Mirex. However, the bioconcentration factor (the ratio of test material concentration in the exposed organism to the concentration of the test material in water) of Mirex was greatest in larvae reared in lower concentrations.

Bookhout et al. (1979) reported that larvae of Rhithropanopeus harrisii reared in Kepone solutions with concentrations as high as 35 ppb exhibited 86 per cent survival through metamorphosis. By comparison, Bookhout et al. (1972) reported increased instar duration and time to metamorphosis, and decreased larval survival when Rhithropanopeus larvae were reared in Mirex solutions with concentrations up to 10 ppb.

Other studies (cited above) which used decapod larvae to assess the toxicity of pesticides reported results similar to those discussed above, i.e., decreased survival of larvae at higher toxicant concentrations and bioconcentration of the pesticide.

To date only one study has been reported in which Kepone-contaminated eggs from field-collected decapods (Palaemonetes pugio) have been reared in the laboratory to determine the affects of the pesticide on larval development (Provenzano et al., 1978). It was concluded that Kepone contained in eggs and adults had no effect on larval survival, larval duration, or length of post-larvae. Experiments were not designed with a priori hypotheses in mind. Samples for Kepone analysis (ovigerous females, females having hatched

eggs, unhatched eggs, newly hatched zoeae, and postlarval shrimp) were pooled by site, making regression analysis of larval development against Kepone content impossible for individual females. The approach used by Provenzano et al. (1978) decreased resolution and reduced the confidence with which conclusions, concerning the relationships between Kepone and larval development, could be made. At the time of the research of Provenzano et al. (1978), the methods for Kepone analysis were not sufficiently refined to analyze small samples (typical of P. pugio) with any degree of confidence.

In an attempt to overcome the problems encountered by Provenzano et al. (1978), blue crabs, which have a larger egg mass than Palaemonetes, were used in the present study. Egg samples for Kepone analysis were not combined for females from the same station, thus the variation due to the female could be accounted for. By the same token, variation in larval development due to parentage could also be accounted for since larvae from individual females were reared separately.

The present study was undertaken to help elucidate whether recent declines in the Chesapeake Bay blue crab population could be related to Kepone. Judging from other studies which used acute bioassay methods to determine the effect of pesticides on decapod larvae, high concentrations of Kepone in blue crabs might be expected to result in 1) low hatchability, 2) an increase in larval duration (thus a greater time to metamorphosis), and 3) a reduction in the number of larvae which complete metamorphosis. Larval abnormalities and reduction in

the size of the "first crab" may also result from high egg-Kepone concentrations.

The present study investigated egg hatchability and larval development of blue crabs, Callinectes sapidus, from Kepone-contaminated eggs. Specific questions addressed were, what effect if any does Kepone contamination in eggs have on hatchability, larval survival, time to metamorphosis, larval morphology, and size of the first crab.

MATERIALS AND METHODS

Sampling

During June and July 1978, crabs were collected from three baited crabs traps left overnight at each of seven stations in Chesapeake Bay (Figure 1). In August the same seven bay stations plus two seaside stations were sampled (Figure 1). Temperature, salinity, and dissolved oxygen measurements were made at each station. Surface temperature was measured at each station using a hand-held thermometer. Surface and bottom water samples from each station were returned to the laboratory for salinity analysis (Beckman induction salinometer, model RS-7B) and determination of dissolved oxygen concentration by a modified winkler titration method (Strickland and Parson 1972).

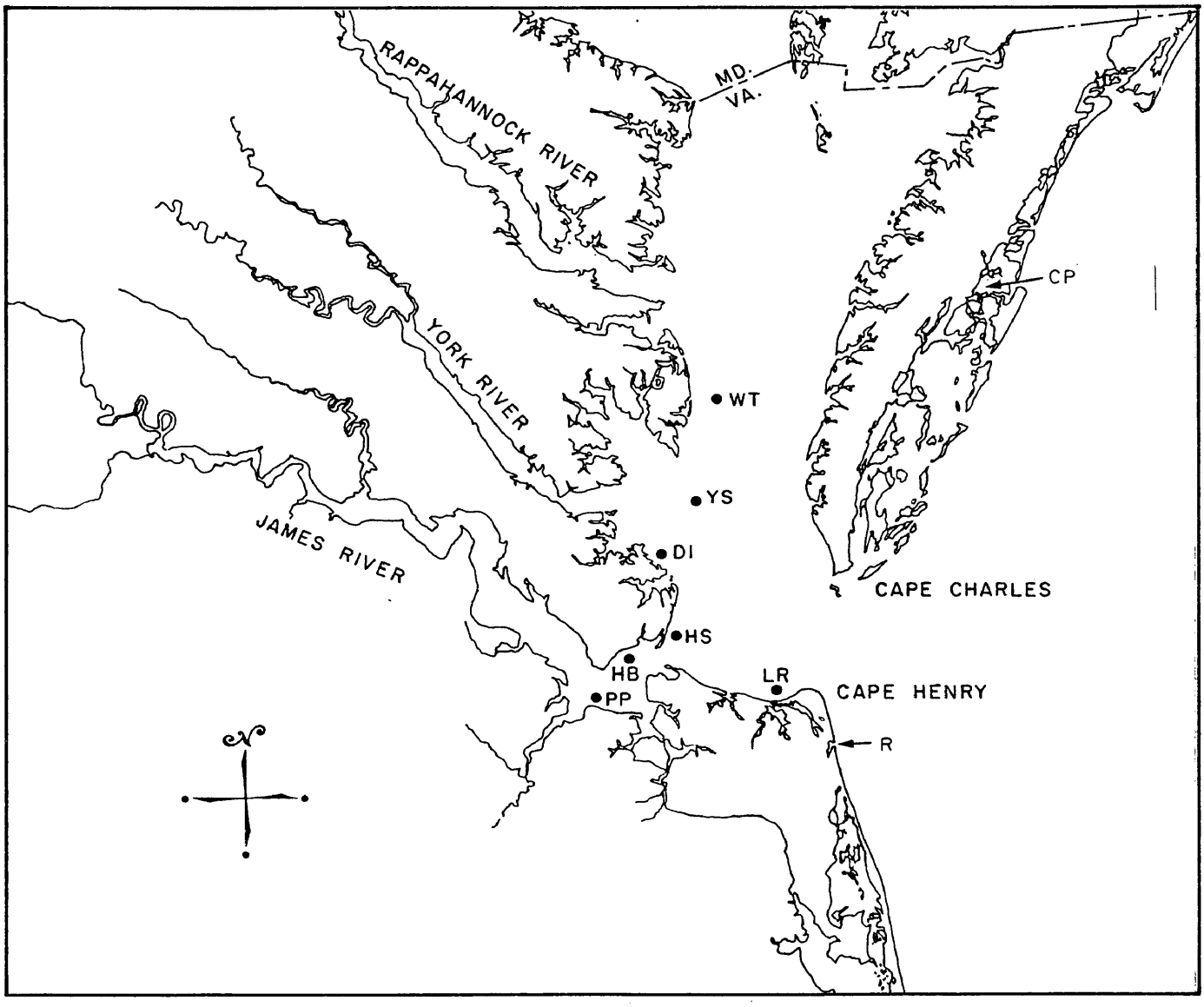
All crabs were categorized as mature males, mature females (the use of these crabs is described in Roberts (1980) and Roberts and Leggett (1980). No immature crabs were retained.

Ovigerous crabs were transported to the laboratory in shallow plastic containers (30.5 X 16.5 X 9 cm), with enough saltwater from the collection site to insure that the egg mass remained moist.

Egg and Larval Culture Media

Culture water was collected from the Eastern Shore field station (Wachapreague Laboratory) of the Virginia Institute of Marine Science. Seawater (approximately 30 $^{o}/_{oo}$) was pumped from Finney Creek and passed through a continuous centrifuge and then through two 1μ

Figure 1. Chart of the Virginia portion of Chesapeake Bay showing sampling site locations. WT - Wolf Trap, YS - York Spit, DI - Drum Island Flats, HS - Horseshoe Flat, LR - Lynnhaven Roads, HB - Hampton Bar. PP - Pig Point, R - Rudee Inlet, and CP - Bradford Bay.


RAPPAHANNOCK RIVER
MD.
VA.
YORK RIVER
JAMES RIVER
CP
WT
YS
DI
HS
HB
PP
LR
CAPE CHARLES
CAPE HENRY
R

cartridge filters arranged in series. Filtered water was collected in 50 gallon plastic barrels which were transported to the laboratory. In the laboratory the water was refiltered through two fresh 1 μ filters and returned to the 50 gallon barrels for storage.

Except for four days in June and four days in July all culture water used was 30 or 31 °/oo. During June and July a barrel of 22 °/oo and 21 °/oo water, respectively, was mistakenly used for the culture of eggs and larvae. In June all eggs but those from crabs HB 2, HB 6, HB 9, PP 1, and YS 2 were cultured in water of 22 °/oo, for at least two days, while all larvae were cultured in this water for at least five days. In July all but the eggs from WT 4 were cultured in 21 °/oo water for at least four days while all larvae were cultured in this water for at least four days.

The medium for egg culture contained penicillin G at 100,000 USP units/l to reduce bacterial and fungal infection (Roberts, 1972). Larval and food culture media contained no bactericides.

Laboratory Processing

Approximately 200 eggs were removed from inside the egg mass of each ovigerous crab. Eggs from beneath the surface of the "sponge" are less likely to have attached parasites. The eggs from each female were placed in individual watch glasses containing egg culture medium. Within each watch glass the eggs were teased apart into smaller clusters and rinsed twice in fresh medium. The aliquot of eggs was divided in half (each portion retaining the crab identification code),

and each portion was placed separately in 20 ml of egg culture medium contained in a plastic compartmented box. The compartmented box was placed on an Eberbach shaker table with a shaking rate of 80 to 100 cycles per minute.

The remainder of the sponge (excluding the pleopods but including setae) from the ovigerous female crab was individually wrapped in aluminum foil, labelled, and frozen for subsequent Kepone analysis.

The year class was determined for all ovigerous females. Additional methods concerned with crab dissection for determination of Kepone partitioning within male, female, and ovigerous female crabs, can be found elsewhere (Roberts, 1980; Roberts and Leggett, 1980).

Kepone Analysis

Blue crab eggs and body tissues were analyzed for Kepone by the Virginia Institute of Marine Science Department of Ecology Pollution. Only a brief discussion is given here concerning the procedures for Kepone analysis. More detailed explanations on the methodology involved in Kepone analyses are found elsewhere (Hodgson et al., 1973; Moseman et al., 1977). Samples were thawed, weighed, and desiccated with a 9:1 mixture of Quso® G30 (precipitated silica) and sodium sulfate. The desiccated sample was homogenized, and Kepone was extracted in a Soxhlet extractor for 16 to 18 h in a 1:1 mixture of petroleum ether and ethyl ether. To remove possible components that might interfere with Kepone measurements the extracted sample was rinsed with a series of solvents through a Florisil column. The

purified sample was analyzed for Kepone by electron capture gas chromatography.

Egg Culture

On the day following collection and initial processing, eggs were examined to determine the degree of development. The criteria used to determine the degree of development were modified after those of Roberts (1972).

After examination for developmental progress and parasites, eggs were counted and transferred to fresh medium in clean compartmented boxes (cleaned with Alconox, a biodegradable surfactant/detergent, and rinsed with tap water and egg culture medium before use) and replaced on the shaker table.

On each subsequent day until hatching, eggs were staged, examined for parasites, and transferred to fresh culture medium in clean compartmented boxes. Constant shaking was administered until the eggs hatched.

Initially it was thought that hatching would be nearly complete 24 h after the first hatch. This method was therefore used for eggs collected in June, i.e., egg culture was terminated 24 h after the first hatch and the number that hatched in the two consecutive days was tallied. The assumption above proved incorrect since per cent hatch was low with many viable eggs left unhatched. In July and August eggs were incubated until no more eggs hatched.

Per cent hatch was determined from the number of larvae that hatched (prezoeae and normal state I zoeae) during the incubation period. Hatched larvae from the same female were combined in an eight-inch culture bowl and only photopositive larvae (those which were attracted to a light source) were placed in culture. Sixty photopositive larvae from each female were divided equally among three, four-inch diameter culture bowls. If the number of hatched larvae on the day of the first hatch numbered less than 60, then bowls of 20 were filled first. The remaining larvae were then placed in a separate bowl. Bowls with less than 20 larvae were discarded only if a bowl of 20 larvae was obtained from a subsequent days hatch. Prezoae were usually abundant but were not included in the cultures because they are not reported to molt to a normal stage I zoeae after shedding the embryonic cuticle (Sandoz and Rogers, 1944).

Photopositive larvae in excess of 60, nonphotopositive larvae, and prezoeae were preserved and examined later for morphological anomalies.

Sufficient rotifers (Brachionus plicatilus) were added to each dish of larvae to yield 40 rotifers per ml of larval culture medium (see below for handling of food cultures). The specimen code for each female plus an additional bowl code was assigned each four-inch culture bowl. Unhatched eggs were examined, transferred to clean egg culture medium, and replaced on the shaker table.

Larval Food Culture Maintenance

A rotifer diet for the first and second larval instars was used because it allowed greater survival to the third instar than a diet of sea urchin (Lytechinus variegatus) gastrulae (Sulkin and Epifanio, 1975). Artemia nauplii are too large for the first and second larval instars to handle. Costlow and Bookhout (1959) first reared the early stages of the blue crab on a mixed diet of sea urchin (Arbacia punctulata) gastrulae and Artemia nauplii. Blue crab larval development from the third instar through metamorphosis is successful using a diet of newly hatched Artemia nauplii.

Rotifers were cultured in a series of two liter beakers. The rotifer diet consisted of unicellular green algae (Chlorella sp.) at a concentration of approximately 10^7 cells per ml of larval culture medium. A 40-liter batch of Chlorella was grown in enriched pasteurized seawater exposed to constant illumination at room temperature. The methodology for algal culture has been described elsewhere (Dupuy et al., 1977).

The protocol for a two beaker rotifer culture system required harvesting 250 ml of a two liter culture every other day. The cultures were harvested on alternating days so that when one culture was harvested, the population in the other beaker was rebuilding to preharvest densities. On a daily basis each culture was rinsed through a 50 μ screen, with waste materials and unused algae being washed free of the rotifers. New medium (1500 ml of larval crab medium and 500 ml Chlorella stock) was prepared and the cleaned

population was washed back into a clean beaker. The density in the rotifer cultures fluctuated between 400 and 900 rotifers per ml on any given day.

Artemia cysts (San Francisco Bay brand) were hatched in inverted one liter I.V. bottles containing larval culture medium. Constant aeration was supplied. Artemia cultures were also harvested on alternate days so that a new batch of nauplii was used each day. New cysts were used to start all cultures. Three dry ml of Artemia cysts per I.V. bottle yielded 1 to 2 X 10^5 nauplii after 48 h of incubation.

Larval Culture and Handling

Larvae were fed 40 rotifers per ml of larval culture medium daily through the second instar. A mixed diet of rotifers and newly hatched Artemia nauplii was added to bowls which contained a mixture of the second and third instars. When all larvae had reached the third instar an exclusive diet of Artemia nauplii was used. All larvae were counted and transferred daily to new medium containing fresh food. Dead larvae were preserved for later examination for morphological anomalies. Larvae were staged according to Costlow and Bookhout (1959).

Bowl cultures were maintained at room temperature with an approximate photoperiod of 14 hours light and 10 hours dark.

Data Presentation and Analysis

For graphical presentation the pertinent data are presented in the form of 'box-and-whisker' diagrams (Tukey, 1977). Box-and-whisker

diagrams were thought to be the best method for presentation of skewed data. Depicted in the box and whisker diagrams are the mean, median, first and third quartiles, and the range of observations. The value presented for larval survival represents the arithmetic mean for replicate bowls.

All percentage values used in the determination of a regression equation and a product-moment correlation coefficient were transformed using the arcsine square-root transformation. Bartlett (1947) suggested the arcsine transformation when analyzing percentages and proportions because it yields a more constant variance.

Regression analysis and significance testing were done for various combinations of the data. A regression equation and product-moment correlation coefficient were also determined. Only pertinent scattergrams for the analyses are included here. The reader is referred to Sokal and Rohlf (1969) for additional discussion on regression and correlation.

RESULTS

Environmental Data

Mean temperature for the three months of sampling was typical for the period (Table 1). The dissolved oxygen concentration fluctuated little and with no pattern over the sampling period (Table 1). Salinity values fluctuated somewhat as a function of location within the Chesapeake Bay (Table 1). Surface and bottom salinity were similar except at Lynnhaven Roads during June when the surface salinity was 19.8 °/oo and bottom salinity was 27.6 °/oo.

Egg Kepone

The concentration of Kepone in blue crab eggs during the present study varied over the sampling period (Figure 2). The median egg Kepone concentration was 0.085 μg/g (mean = 0.23 μg/g) in June, 0.31 μg/g (mean = 0.35 μ/g) in July, and 0.01 μg/g (mean = 0.08 μg/g) in August. In addition to being variable, egg Kepone levels were consistently skewed towards low concentrations.

For purposes of a later discussion, stations were classified as lower James (LJ) (Hampton Bar and Pig Point), lower bay (LB) Lynnhaven Roads, Horseshoe Bar, York Spit, and Wolf Trap), or ocean (O) (Rudee Inlet and Bradford Bay in the Wachapreague Inlet) (Figure 3). No ovigerous female crabs were collected at Drum Island Flats during the study period. Median and mean Kepone concentrations tended to be higher in crabs from the lower James in July and August than those from the lower bay and ocean. In June the median and mean egg Kepone

TABLE 1

Surface and bottom ( ) salinity and dissolved oxygen, and surface temperature for the months of sampling at the various stations (see captions, Figure 1, for station codes). - no sample collected, + sample lost.

Station	June	July	August
		Salinity (°/oo)	
HB	15.5(15.8)	19.6(19.7)	+(+)
HS	18.3(18.3)	19.0(20.5)	+(+)
LR	19.8(27.6)	19.8(19.8)	+(+)
PP	13.2(-)	16.7(16.8)	+(+)
WT	11.3(13.4)	14.3(15.9)	17.2(19.1)
YS	14.6(17.3)	16.7(17.4)	19.1(19.6)
CP	-(-)	-(-)	30.0(-)
R	-(-)	-(-)	-(-)
		Dissolved Oxygen (mg/1)	
HB	5.3 (4.1)	6.9 (6.5)	+(+)
HS	4.9 (4.2)	7.1 (4.7)	+(+)
LR	4.8 (3.9)	+(+)	+(+)
PP	4.3 (3.4)	6.1 (6.1)	+(+)
WT	3.6 (3.9)	0.5 (0.5)[1]	4.5 (3.7)
CP	-(-)	-(-)	-(-)
R	-(-)	-(-)	11.8(-)[2]
		Temperature (°C)	
HB	+	23	+
HS	23	24	+
LR	22	+	+
PP	22	24	+
WT	22	23	27
YS	22	23	27
CP	-	-	-
R	-	-	29

[1]Low value reflects sampling error. Sample included some anaerobic bottom sediments.

[2]High value is unexplainable.

Figure 2. Kepone concentration of eggs from ovigerous crabs for all sampling stations grouped by sampling month. n = sample size.

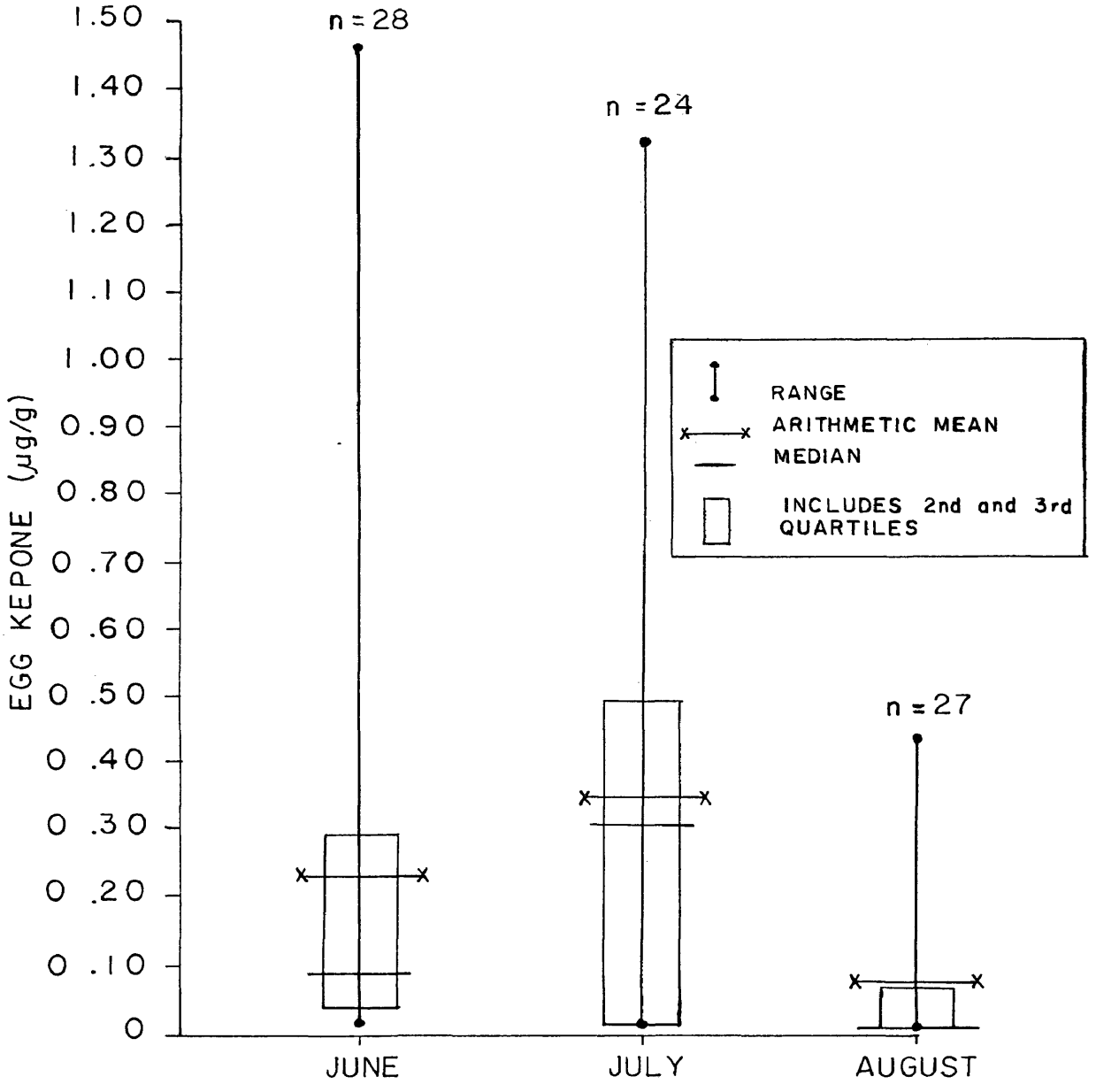
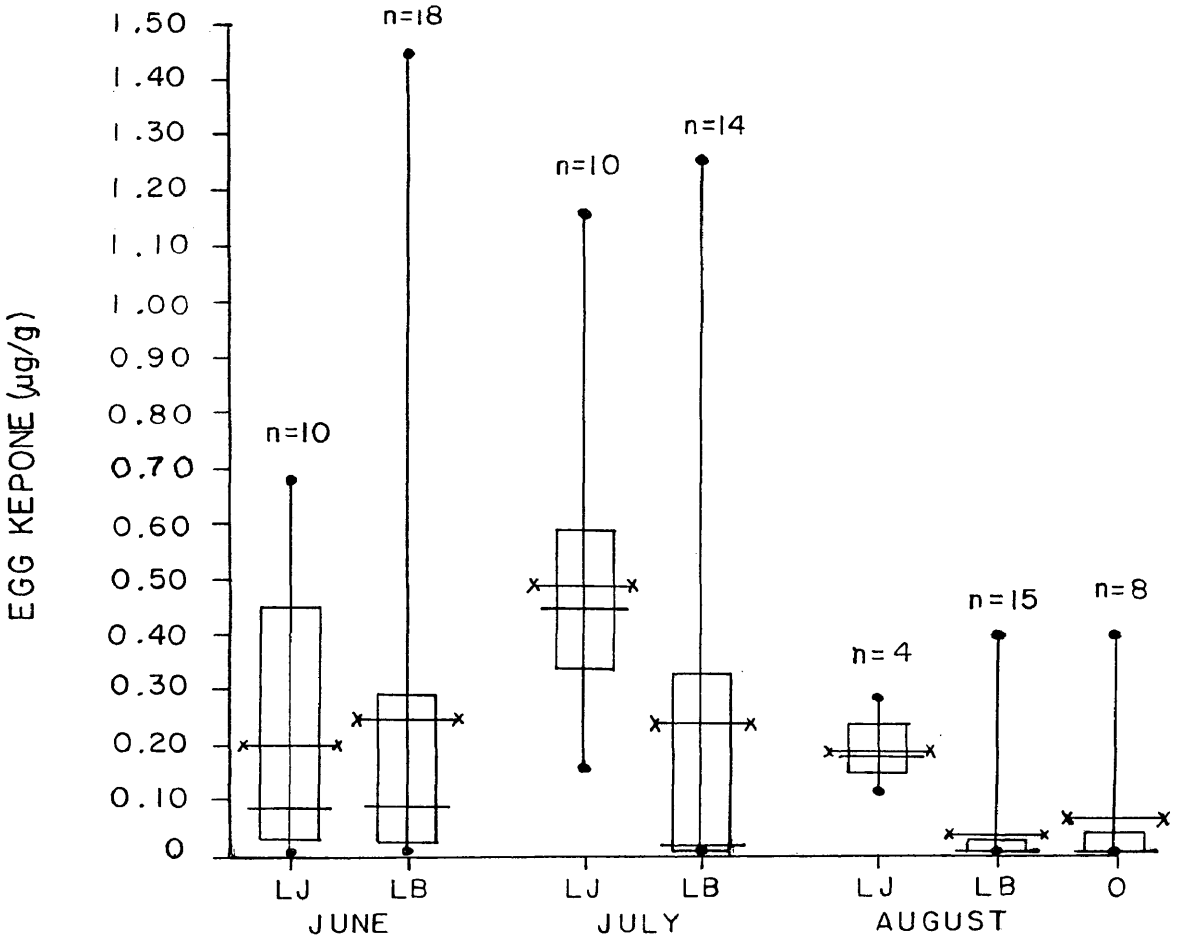

1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
EGG KEPONE (µg/g)
n = 28
n = 24
n = 27
RANGE
ARITHMETIC MEAN
MEDIAN
INCLUDES 2nd and 3rd QUARTILES
JUNE
JULY
AUGUST

Figure 3. Kepone concentration of eggs from ovigerous female blue crabs aggregated by location in the lower Chesapeake Bay (LB), lower James River (LJ), and ocean (O). n = sample size (symbols as in Figure 2).


EGG KEPONE (µg/g)
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
n=10
n=18
n=10
n=14
n=4
n=15
n=8
LJ
LB
LJ
LB
LJ
LB
0
JUNE
JULY
AUGUST

concentrations were only slightly higher in lower Bay crabs than crabs from the lower James.

Examination of the egg Kepone data in Appendix A reveals considerable variability among stations as was expected. Egg Kepone concentrations tended to be highest in crabs collected in the vicinity of the James River (HB, PP, and HS), except in August during which ovigerous female crabs from Horseshoe Bar carried eggs containing very low concentrations of Kepone. By comparison the crabs from Lynnhaven Roads, Wolf Trap, Bradford Bay, and Rudee Inlet carried eggs containing low concentrations of Kepone. Further examination of Appendix A reveals substantial variability of egg Kepone concentration among crabs from the same station during all sampling months.

In summary, egg Kepone concentration tended to be highly variable in space and time and was heavily skewed toward low concentration. Concentrations among crabs from the same station were low with only a few crabs carrying eggs with a significant quantity of Kepone. Roberts and Leggett (1980) considered only ovigerous female blue crabs carrying eggs containing 0.01 μg/g Kepone to be significantly contaminated. According to their criterion, the majority of the ovigerous crabs collected in the present study were carrying uncontaminated eggs (Figures 2 and 3). Further statistical analysis was precluded by the skewness and variability of the data.

Hatchability and Egg Kepone

Analysis of the hatchability data revealed an apparent loss of eggs during the incubation period. The number of eggs initially placed in culture for 58 of 73 crabs was greater than that which could be accounted for at the termination of the incubation period. Per cent $hatch_{(I)}$ in Appendix A is based on the egg count made at the beginning of the incubation period, while per cent $hatch_{(A)}$ is based on the number of eggs which could be accounted for at the termination of the incubation period (% $hatch_{(I)}$ smaller than per cent $hatch_{(A)}$ when eggs were lost). A gain in eggs simply means that more eggs were accounted for at the termination of the incubation period than at the beginning. The number of eggs lost or gained plotted against time in culture yielded negative slopes. Only the correlation of July was significant ($r = -0.519$, $\alpha = 0.05$). The percentage of eggs lost per day was determined to be 0.017, 0.88, and 0.04 for June, July, and August, respectively (calculated from the slope of the regression line of $\text{Sin}^{-1}(\%\ \text{Loss/Gain})^{1/2}$ plotted against time in culture. Only July had significant loss (b significantly different from zero, $\alpha = 0.05$).

When monthly transformed per cent hatch data were plotted against total time in culture, only the data for July had significant correlation ($r = -0.585$, $\alpha = 0.01$). All slopes were negative (apparently a decrease in per cent hatch with increased duration of culture) but only the July slope was significant ($\alpha = 0.05$).

Thus there is only a slight indication that optimal culture conditions were not maintained throughout the egg incubation period.

Factors responsible for egg loss include deterioration by bacterial or fungal infection, inadequate handling procedures, or inaccurate counting at the beginning and/or end of incubation.

To evaluate the possiblity that egg Kepone concentration may have been responsible for egg loss (i.e., high levels of Kepone in eggs may have caused death of the eggs, resulting in egg deterioration and finally loss) transformed per cent eggs lost/gained was plotted as a function of egg Kepone concentration for the months of June, July, and August. All slopes were negative and not significantly different from zero ($\alpha = 0.05$). Likewise correlation coefficients were not significant ($\alpha = 0.05$).

Because per cent hatch could be determined using the number of eggs which were initially placed in culture or by using the number of eggs which could be accounted for at the end of the incubation period, both values were plotted against each other to examine the difference between the two (Figure 4). The high degree of correlation between per cent $\text{hatch}_{(I)}$ and per cent $\text{hatch}_{(A)}$ ($r = 0.90$, $\alpha = 0.01$) suggested that either value of per cent hatch would give a reliable estimate of the parameter. For purposes of the present paper, per cent $\text{hatch}_{(I)}$ was used for the remaining analyses.

Box and whisker diagrams were used to present the hatchability data graphically. Egg hatchability was low and highly variable throughout the study (Figure 5). Overall there was a slight increase in hatchability after June (Figure 5). The increase in hatchability

Figure 4. The arcsine transformation of the square root of the per cent hatch$_{(I)}$ plotted against the arcsine transformation of the square root of per cent hatch$_{(A)}$ for all three sampling months.

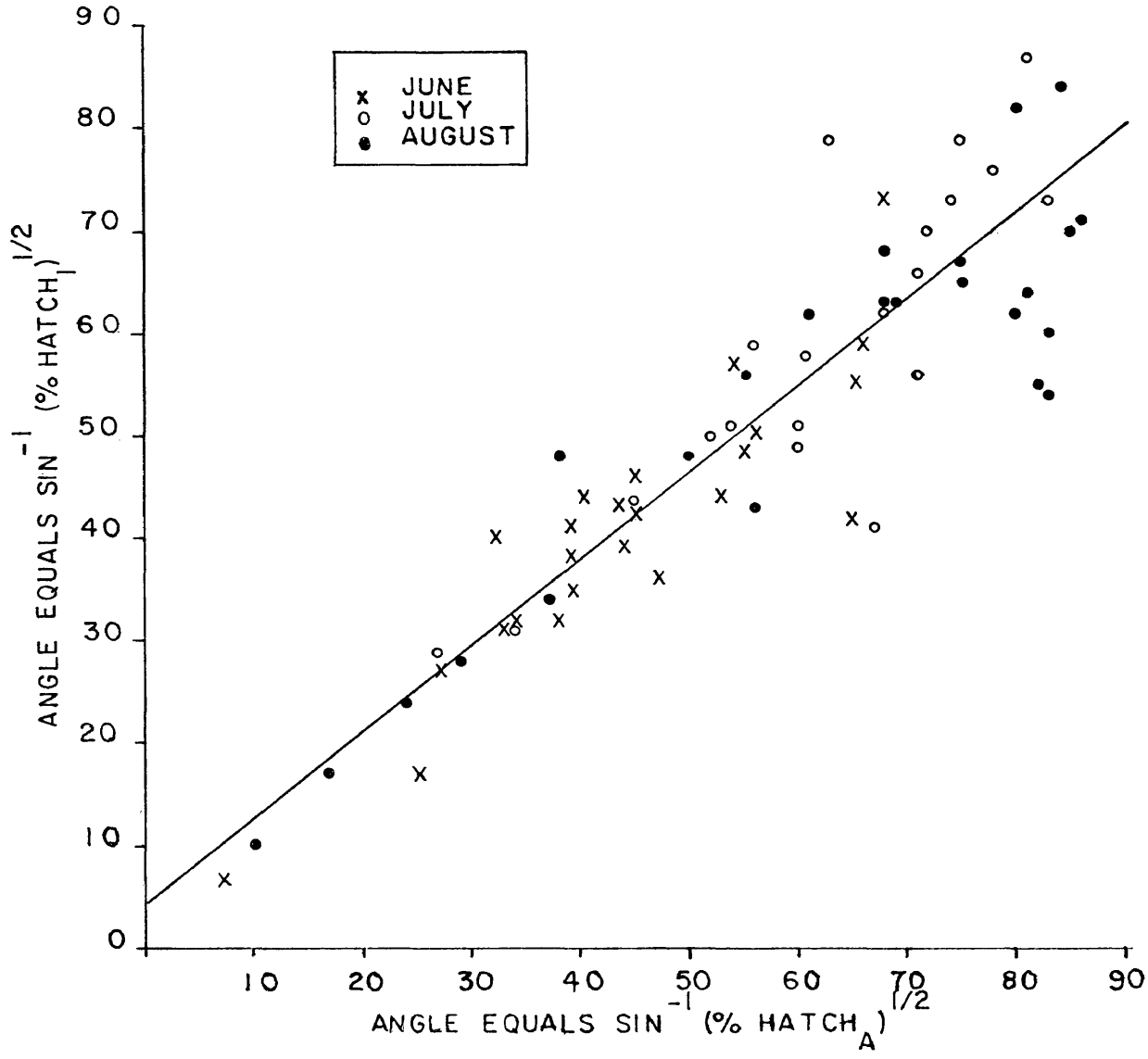
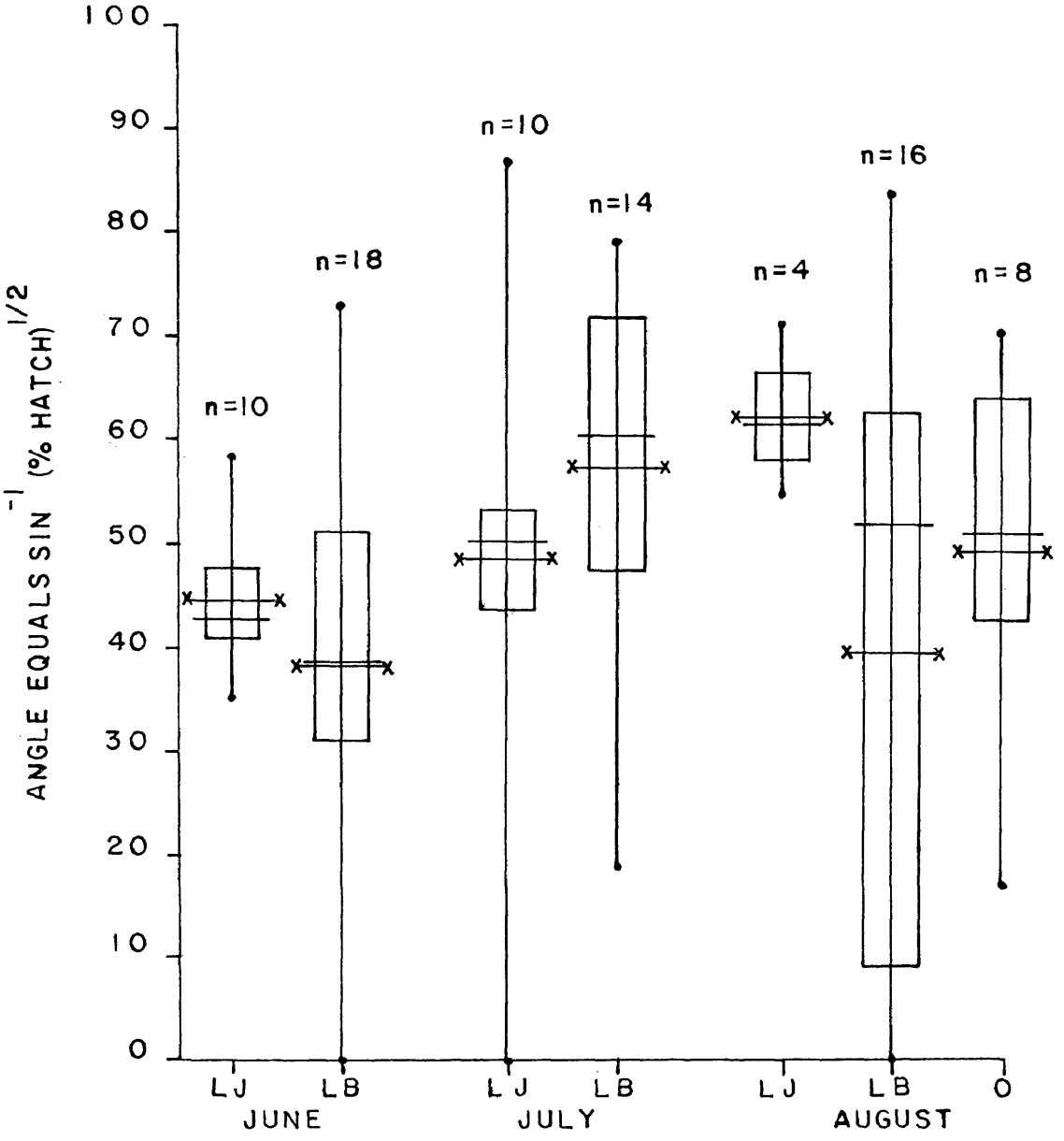

JUNE
JULY
AUGUST
ANGLE EQUALS SIN^{-1} (% HATCH$_I$)$^{1/2}$
ANGLE EQUALS SIN^{-1} (% HATCH$_A$)$^{1/2}$
0
10
20
30
40
50
60
70
80
90

Figure 5. The arcsine transformation of the square root of the per cent hatch aggregated by location in lower Chesapeake Bay (LB), lower James River (LJ), and ocean (O), grouped by sampling month. n = sample size (symbols as in Figure 2).

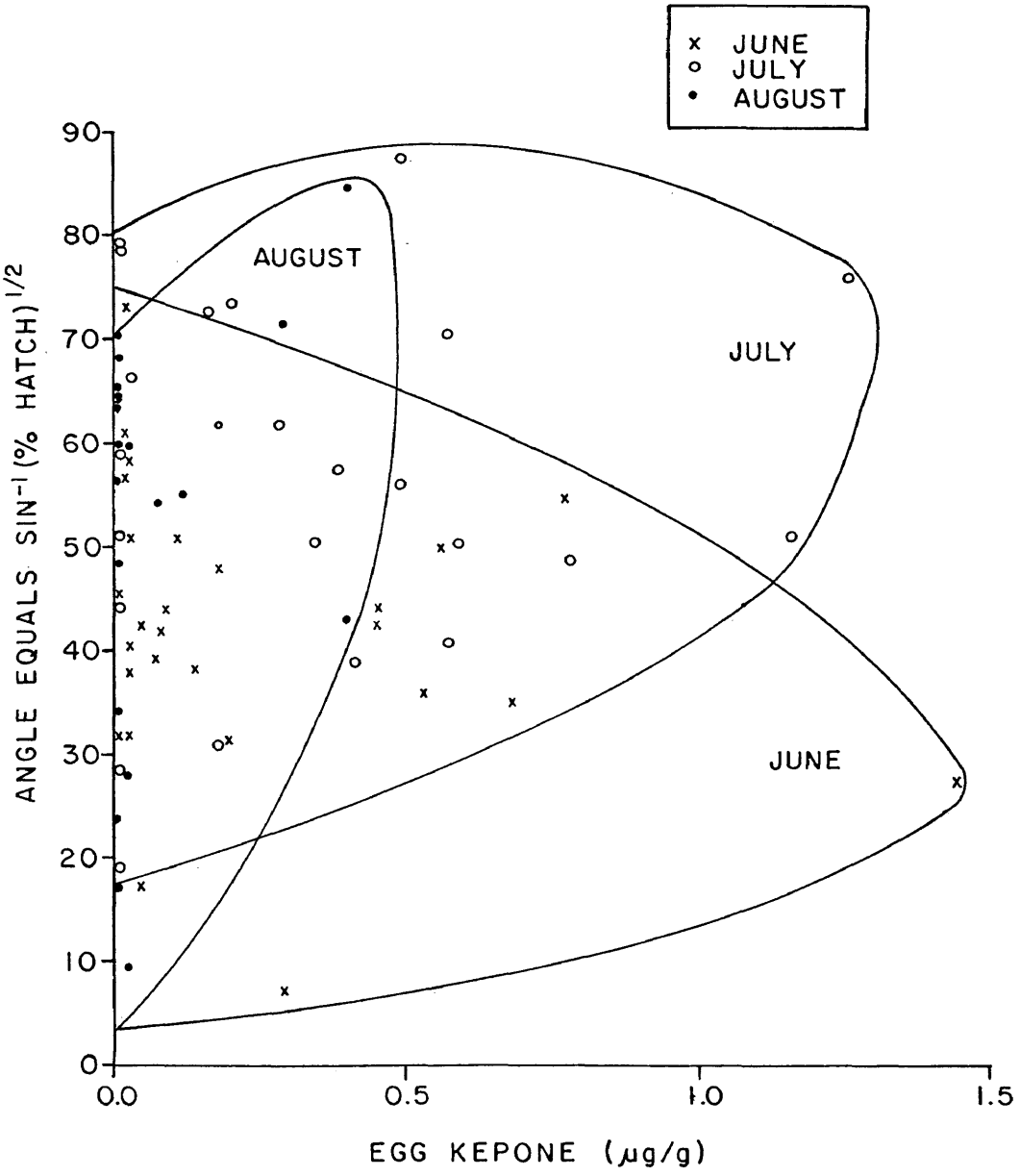

ANGLE EQUALS SIN $^{-1}$ (% HATCH)$^{1/2}$
100
90
80
70
60
50
40
30
20
10
0
n=10
n=18
n=10
n=14
n=4
n=16
n=8
LJ
LB
LJ
LB
LJ
LB
O
JUNE
JULY
AUGUST

in July reflects the change in culture methodology, while the change in culture media may reflect the overall slight increase in hatchability in August.

Lower James River stations were grouped as described earlier on the assumption that crabs from these stations would have lower per cent hatch values relative to lower Chesapeake Bay and ocean crabs. The assumption above was not demonstrable. On the contrary, ovigerous crabs from the lower James yielded a higher per cent hatch during June and August. Only lower James crabs in July carried eggs which hatched with less success than lower Chesapeake Bay crabs. It is doubtful that the low per cent hatch values obtained during July from lower James crabs stemmed from high egg Kepone levels. The apparent relationship was more likely spurious since the results from June and August were nearly opposite.

There was no significant correlation between transformed per cent hatch and egg Kepone concentration ($\alpha = 0.05$). These results are contrary to the expectation that egg Kepone would result in decreased hatching success. The low per cent hatch values in June which resulted from the culture protocol coupled with many low values for egg Kepone concentration accounts for the negative slope observed for the June data. Scattergrams of transformed per cent hatch plotted against egg Kepone concentration are presented in Figure 6 for each month. Regression lines are not included but the data for each month are encompassed by a line fitted by eye.

Figure 6. The arcsine transformation of the square root of the percentage hatch plotted as a function of egg Kepone concentration (μg/g). Sampling months are encompassed by an eye fitted curved line.


JUNE
JULY
AUGUST
AUGUST
JULY
JUNE
90
80
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
ANGLE EQUALS SIN⁻¹ (% HATCH)^1/2
EGG KEPONE (µg/g)

In an attempt to reveal a relationship between egg Kepone and embryological development time (i.e., that high levels of Kepone may increase embryological duration or suppress it all altogether), the time to first hatch in days was plotted against egg Kepone concentration. There was no effect of egg Kepone on the time to first hatch. In part, this was a result of not placing all eggs in culture at the same point in their embryological development.

There was no significant difference in hatchability of eggs spawned from 1976 and 1977 year class females, as a function of egg Kepone concentration.

To summarize, egg hatchability was low but the observed values were not demonstrably related to egg Kepone concentration. There was also no apparent effect of egg Kepone on embryological development time. The criteria used to establish embryological stage were on too gross a level and the developmental period is too short to resolve any effect of Kepone on developmental time. With the given experimental design there is no way to determine any impact of environmental variables experienced by the crab and eggs on hatchability.

Larval Survival and Egg Kepone

The majority of the larval survival data are presented in tabular form in Appendix B. The majority of the data applies only to prezoeae and zoeal stages I and II. Too few larvae survived beyond the second instar to analyze the data for Kepone effects on later stage larvae. Per cent survival for those crabs which did produce larvae that

survived beyond zoeae II are presented in Table 2. Only two larvae from over 4000 studied survived to the megalopal stage. Had these two larvae survived through metamorphosis (and one of them might have, had it not been accidentally discarded) only 0.05 per cent survival to the first crab would have been achieved. The number of larvae that survived beyond zoeae II increased dramatically with each sampling month. This probably reflects improvement in culture technique and use of higher salinity culture water. Eggs which produced larvae that survived beyond the second instar contained as much as 1.45 μg/g Kepone ($\bar{x}$ = 0.3, Mn = 0.14 μg/g), the highest level in this study (Table 2).

Per cent hatch as prezoeae was plotted as a function of egg Kepone concentration. The data are presented in Appendix B. There was no relationship between the two variables. In fact, July and August data yielded a negative regression line which is contrary to expectation. Correlation coefficients were all nonsignificant (α = 0.05) and the slopes were not significantly different from zero (α = 0.05). The variability in the percentage of prezoeae coupled with the skewed nature of the Kepone data would mask any relationship if it were present.

Per cent hatch as prezoeae plotted as a function of sampling site bottom salinity at the time of sampling revealed no relationship. The July data yielded a non significant negative correlation (α = 0.05). Similarly, the slopes were not significantly different from zero (α = 0.05). June and August data yielded positive regression lines

TABLE 2

Tabulation of those larvae which survived beyond the second zoeal instar and the Kepone concentration of eggs from which the larvae hatched (M = megalopa).

Month	Crab	Highest Instar Reached	Egg Kepone (μg/g)
June	HS5	III	1.45
	LR7	III	0.77
July	HB17	III	0.16
	HS12	III	1.26
	HS16	III	0.57
	HS11	IV	0.28
	HS13	IV	0.01
	PP15	M	0.34
August	HB32	III	0.12
	HB34	III	0.29
	HS24	III	0.01
	HS26	III	0.01
	YS19	III	0.03
	R 2	III	0.01
	HS25	IV	0.01
	WT15	IV	0.40
	LR15	VI	0.01
	CP 2	VI	0.01
	HB33	VII	0.18
	HS27	M	0.01

(slopes not significantly different from zero at $\alpha = 0.05$) with nonsignificant correlation ($\alpha = 0.05$).

Larval survival data are presented graphically as box and whisker diagrams in Figure 7 (zoeae I) and 8 (zoeae II), and in tabular form in Appendix B. Sampling stations are grouped as lower James, lower Chesapeake Bay, or ocean. Crabs from the lower James generally had a higher survival in the first stage than lower Bay crabs (and ocean crabs in August). This is contrary to expectation as one would expect lower James crabs containing higher levels of egg Kepone (except for June (Figure 3)) to yield a lower per cent survival of larvae. On the other hand zoeae II from lower Bay crabs, had a higher per cent survival than lower James crabs and ocean crabs in August. The latter observation is also contrary to expectation as one would expect ocean crabs to have a much higher per cent survival than Chesapeake Bay crabs, since maximum survival under laboratory conditions occurs in higher salinity water (Costlow and Bookhout, 1959).

In general the larval survival data were skewed towards low concentrations. The skewness was not surprising since other workers have reported high mortality during the first few instars of the blue crab (e.g., Costlow and Bookhout, 1959; Sulkin and Epifanio, 1975). Thus the problem of resolving an effect from egg Kepone concentration on larval development is complicated, especially since the egg Kepone concentration data are also skewed.

Figure 7. The arcsine transformation of the square root of the per cent survival of stage I zoeae aggregated by location in lower Chesapeake Bay (LB), lower James River (LJ), and ocean (O), for each sampling month. n = sample size. (symbols as in Figure 2).

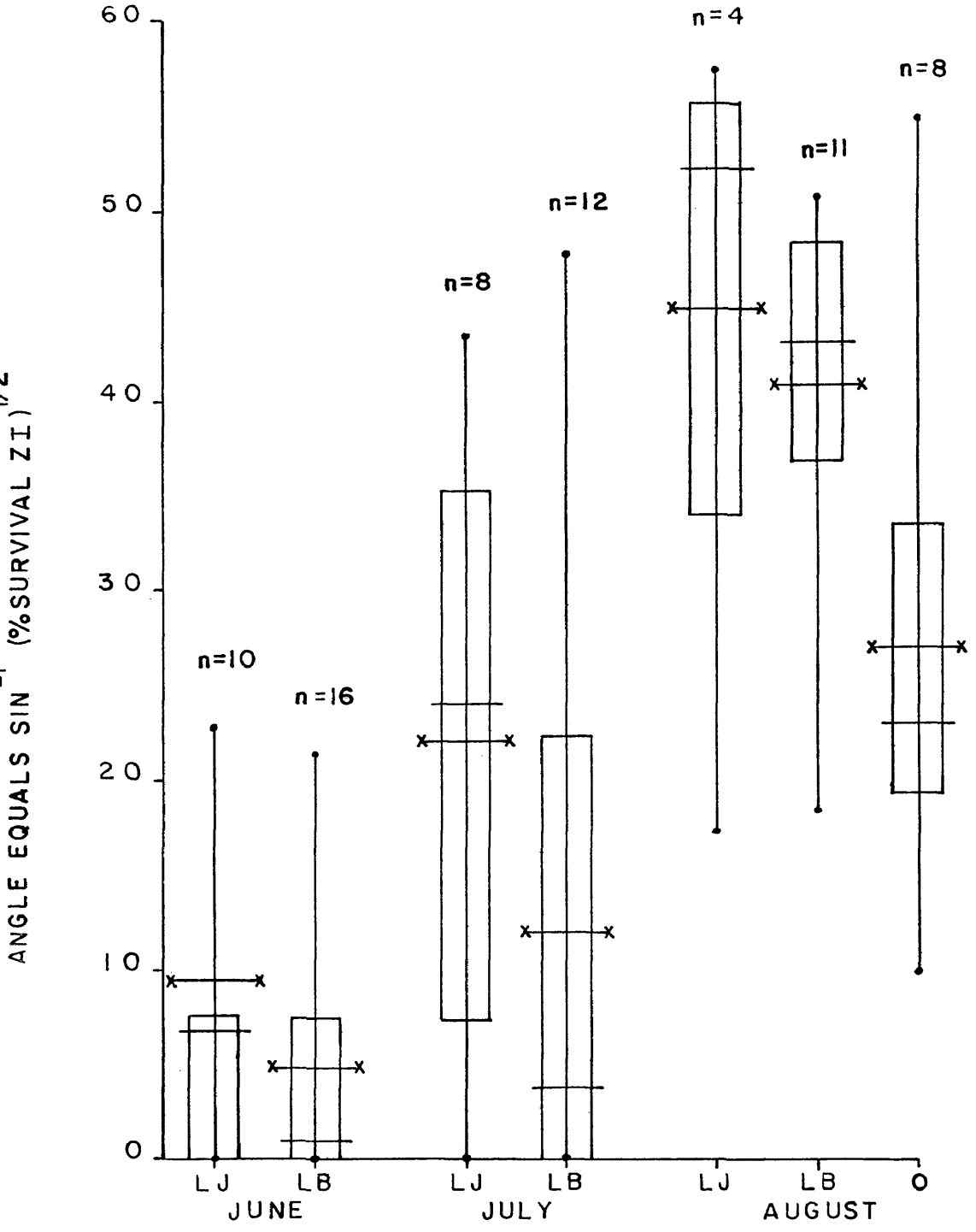
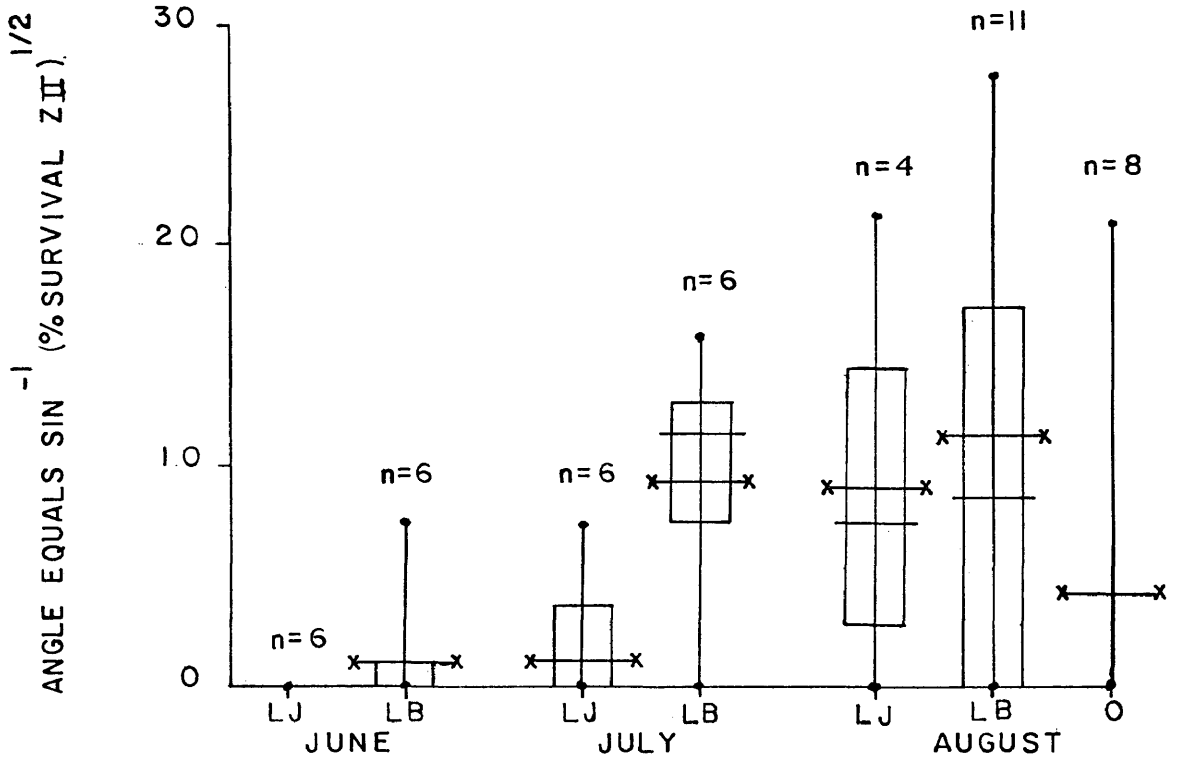

ANGLE EQUALS SIN^{-1} (%SURVIVAL ZI)$^{1/2}$
60
50
40
30
20
10
0
n=10
n=16
n=8
n=12
n=4
n=11
n=8
LJ
LB
LJ
LB
LJ
LB
O
JUNE
JULY
AUGUST

Figure 8. The arcsine transformation of the square root of the per cent survival of stage II zoeae aggregated by location in lower Chesapeake Bay (LB), lower James River (LJ), and ocean (O), for each sampling month. n = sample size. (symbols as in Figure 2).


ANGLE EQUALS SIN -1 (% SURVIVAL ZII) 1/2
30
20
1.0
0
n=6
n=6
n=6
n=6
n=4
n=11
n=8
LJ
LB
LJ
LB
LJ
LB
O
JUNE
JULY
AUGUST

Transformed per cent survival of stage I and stage II zoeae is plotted as a function of egg Kepone concentration in Figures 9 and 10, respectively. In every instance for all three sampling months and both stages, positive slopes (and spurious correlations for June stage I and stage II zoeae) were obtained. The correlation for July and August data was not significant ($\alpha = 0.05$) and slopes of the regression lines for all months were not significantly different from zero ($\alpha = 0.05$).

The mean time to the stage II zoeae was plotted against egg Kepone concentration. There was no significant correlation between the two variables ($\alpha = 0.05$) and the slopes (positive for all three months) were not significantly different from zero ($\alpha = 0.05$).

The maximum number of instars completed for larvae of each ovigerous crab was plotted as a function of egg Kepone concentration. There was no significant correlation between the two variables for July and August data (positive and negative nonsignificant correlation, respectively, $\alpha = 0.05$) but the June data yielded significant correlation ($\alpha = 0.01$). The slope was significant at $\alpha = 0.005$. These results are contrary to expectation.

Nonsignificant positive correlation and slopes ($\alpha = 0.05$) were obtained when the mean time to death was plotted as a function of egg Kepone concentration.

Macroscopic examination of nearly all larvae which hatched in this study revealed no larval abnormalities, barring a few larvae with

Figure 9. The arcsine transformation of the square root of the per cent survival of stage I zoeae plotted as a function of egg Kepone concentration (μg/g) for each sampling month. The data for individual sampling months are encompassed by an eye fitted curved line.

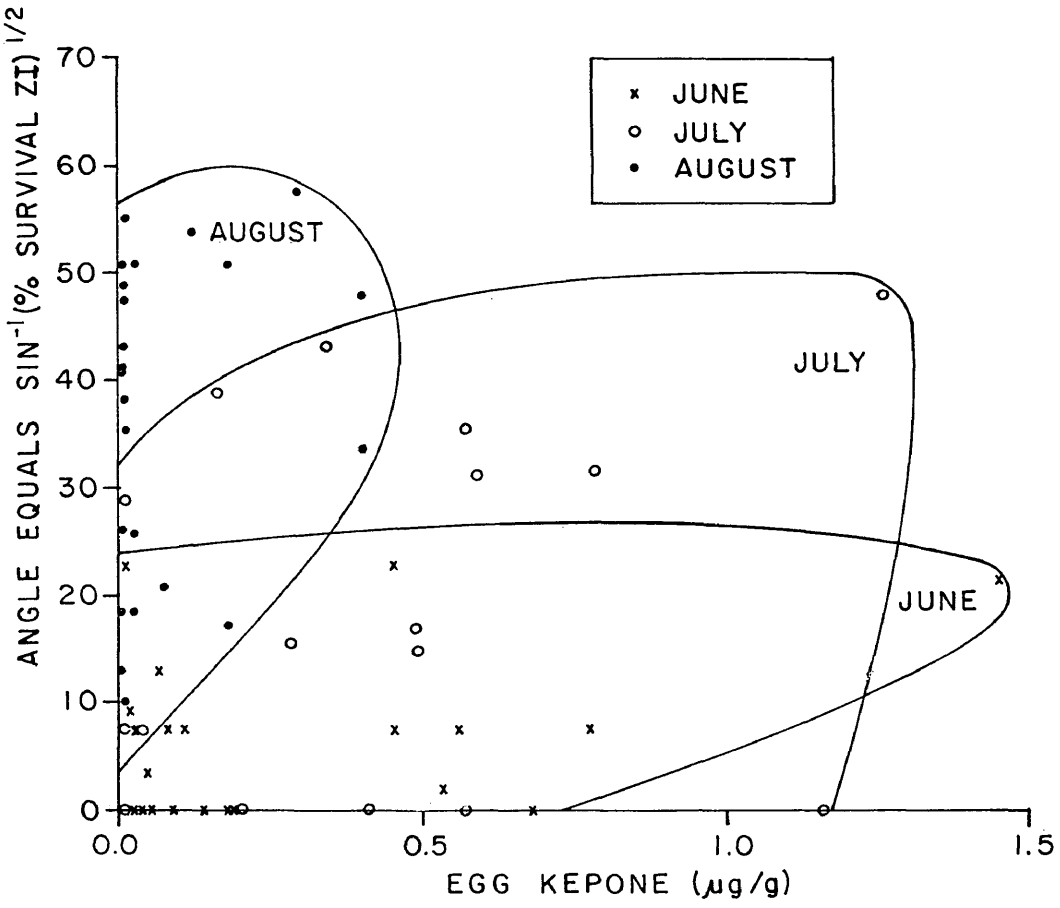
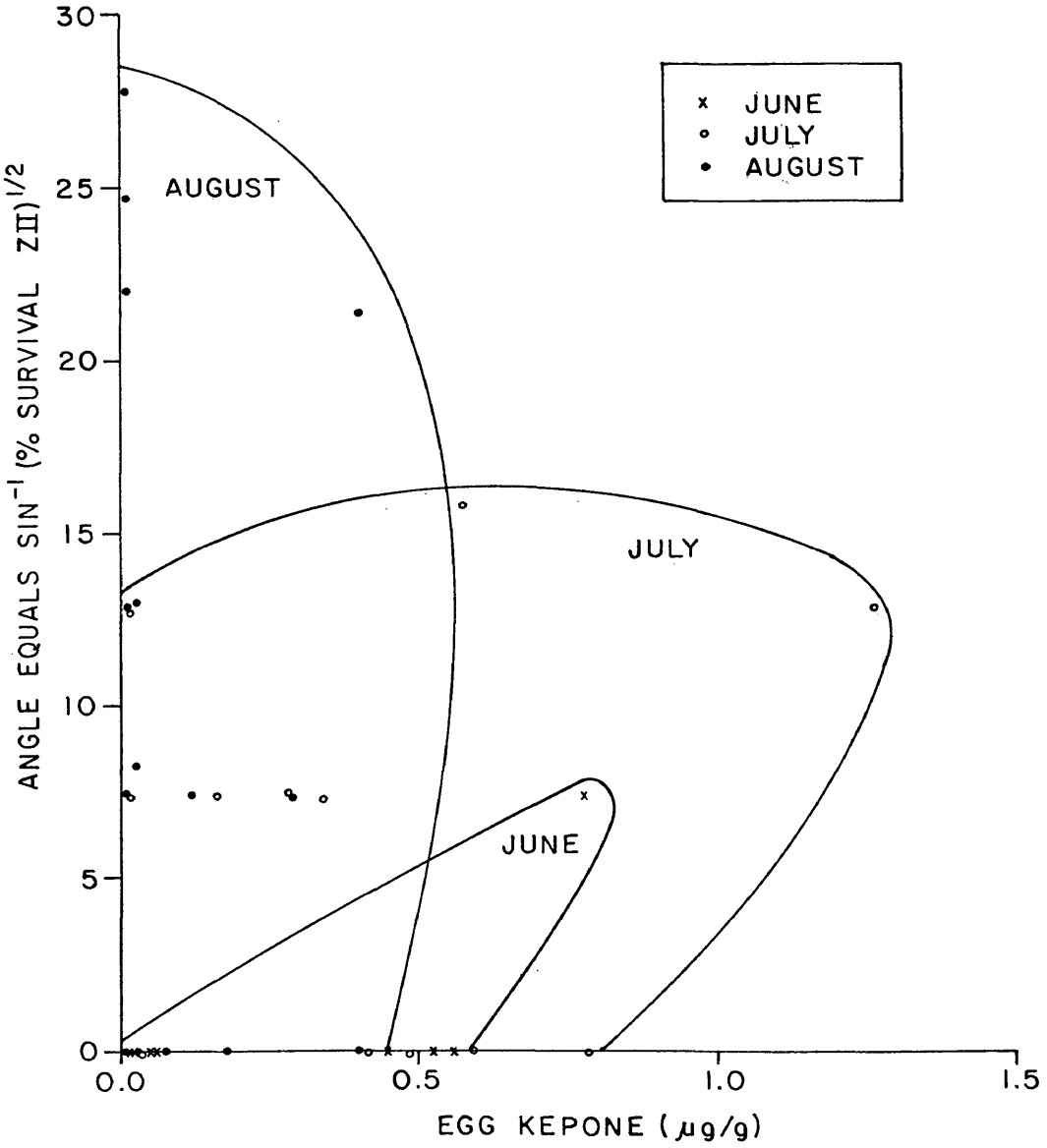

ANGLE EQUALS SIN⁻¹(% SURVIVAL ZI)^1/2
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
EGG KEPONE (µg/g)
JUNE
JULY
AUGUST
AUGUST
JULY
JUNE

Figure 10. The arcsine transformation of the square root of the per cent survival of stage II zoeae plotted as a function of egg Kepone concentration (μg/g) for each sampling month. The data for individual sampling months are encompassed by an eye fitted curved line.


30
25
20
15
10
5
0
0.0
0.5
1.0
1.5
ANGLE EQUALS SIN^{-1} (% SURVIVAL ZII)$^{1/2}$
EGG KEPONE (µg/g)
JUNE
JULY
AUGUST
AUGUST
JULY
JUNE

bent exoskeletal spines. These few abnormalities can be attributed to hardening of the exoskeleton after a spine was bent, or to slow expansion of the larva after molting. In any case, these occurrences were extremely scarce and no attempt was made to correlate them with egg Kepone concentration.

There was no significant difference between larval survival from 1976 and 1977 year class females, as a function of egg Kepone concentration.

In summary, only two larvae from over 4000 laboratory hatched eggs survived to the megalopal instar. One of these was accidentally discarded and the other died prior to the molt to juvenile. The majority of larval mortality occurred in the first two instars. Low survival rate was not demonstrably related to egg Kepone concentration. Similarly, egg Kepone concentration was not demonstrably responsible for the appearance of prezoeae, nor did Kepone have an effect on the developmental time to the second instar, the number of instars completed, the mean time to death, or the external morphology of larvae.

DISCUSSION

Egg Kepone

It is difficult to explain the variable egg Kepone levels in crabs from the same station. Furthermore, no substantial explanation can be offered as to why egg Kepone levels varied over the three month sampling period. Similar variability was observed over time in blue crab backfin muscle samples collected and analyzed by the Bureau of Shellfish Sanitation, Virginia Department of Health (see Roberts and Leggett, 1980). In addition to being highly variable, egg Kepone concentrations are not normally distributed. Just speculating, one may attribute intrasite variation in egg Kepone levels to short-term movements of the female crab. While seasonal migrations are reported in the literature (e.g., Van Engel, 1958), quantitative information is lacking on short-term movements, i.e., day-to-day or week-to-week movements. Perhaps ovigerous crabs routinely travel within the salinity regime which is required for eggs to remain viable, and at the same time accumulate Kepone. A more realistic explanation would be that blue crabs eat a wide variety of food material, much of which is migratory, and already contains Kepone.

Blue crab eggs from the vicinity of the James River were expected to contain higher levels of Kepone than crabs collected in other areas of the Chesapeake Bay. However, only July and August crabs from the James River carried eggs which contained higher concentrations of Kepone than crabs from other areas of the Chesapeake Bay. Data from Roberts (1980), Roberts and Leggett (1980), and from the Virginia

Bureau of Shellfish Sanitation (in Roberts and Leggett, 1980) indicate that James River female blue crabs are more contaminated with Kepone than female crabs from other areas of the Chesapeake Bay.

The very pronounced variability and the skewed pattern of egg Kepone levels made it difficult to uncover a relationship between egg Kepone and blue crab development. One of the underlying assumptions for most statistical analyses (e.g., ANOVA, linear regression, and correlation analysis) is that the data be normally distributed with common variance between random samples (Steele and Torrie, 1969). With this assumption not met in the present data, conclusions drawn from statistical treatment of the data would not be reliable. Even data transformation yielded little improvement in the correlations and significance of the slopes. In fact those correlations and slopes that were significant must be carefully scrutinized because of the nature of the data.

Hatchability and Egg Kepone

One explanation for loss of eggs in 58 of 73 ovigerous crabs is that egg handling procedures were inadequate. It was assumed that the eggs were firmly attached to the setae of the pleopods. In all likelihood eggs were left behind in the compartments during transfer procedures. Although there are no data to verify this speculation, it seems to be the only reasonable way in which eggs could be lost in the numbers reported. Another route for egg loss is egg deterioration. This was very prominent in a number of egg batches, especially those from Lynnhaven Roads in August. When deterioration was observed,

heavy sloughing of eggs from the sponge had occurred, and a poor hatch followed. The eggs were usually uncountable and indistinguishable from a detrital mass. Some of this condition may have been a result of the high water temperatures (27 to 29°C) which occurred in August. The high temperatures may have promoted rapid development of bacterial and fungal growth. Sandoz and Rogers (1944) reported high frequency of occurrence of this condition in crabs collected from Hampton Bar. Table 3 presents data for those crabs whose eggs either sloughed off or developed large quantities of epiphytic growth. Accompanying these data is the bottom salinity at the time of crab collection, per cent hatch, per cent prezoeae, and the egg kepone concentration. There is no apparent relationship between these variables. Though high levels of egg Kepone could kill the eggs, thus rendering them subject to bacterial or fungal infection, sloughing, and finally loss, it was demonstrated earlier that no such relationship exists in the present data. For example, crab HS 12 and a high egg Kepone concentration (1.26 µg/g), but no sloughing or epiphytic growth was observed and there was only 7.3% egg loss. Crab R1 by comparison (Table 3) had only 0.04 µg/g egg Kepone, but exhibited epiphytic growth and high loss. Thus it might be concluded that the loss of eggs was a result of poor handling technique. The loss on a daily basis was negligible, as was pointed out earlier.

Apparent gain in eggs can be attributed to counting error, and in those cases where a substantial increase is reported, to carry-over from one compartment to the next.

TABLE 3

Crabs which had sloughing eggs (*) and/or epiphytic growth (+) on cultured eggs.

Month	Crab	Egg Kepone (µg/g)	Bottom Salinity (°/oo)	Sloughing	Epiphytic Growth	% Loss-Gain	% Pre-zoeae	% Hatch
June	LR 6	0.29	27.57		+	- 3.40	100.0	1.67
July	HB19	0.34	19.68		+	-	-	0.00
	HS10	0.01	20.50		+	- 6.80	53.4	60.47
	HS15	0.57	20.50		+	- 1.60	2.4	88.85
	PP15	0.34	16.84		+	- 8.50	10.1	59.60
	YS 5	0.01	17.43		+	3.20	0.0	10.57
	YS 6	0.01	17.43		+	- 2.30	38.8	45.59
August	HB32	0.12	NS[1]	*		-31.70	20.4	67.07
	HS23	0.01	NS[1]	*		- 2.83	74.4	68.91
	HS26	0.03	NS[1]		+	-13.90	0.0	74.45
	LR16	0.01	NS[1]	*	+	48.70	100.0	55.78
	LR17	0.03	NS[1]	*	+	- 6.60	94.6	21.88
	LR18	0.05	NS[1]		+	-	-	0.00
	LR19	NS[1]	NS[1]	*	+	ND[2]	0.0	1.28
	LR20	0.04	NS[1]	*	+	-	-	0.00
	LR21	0.01	NS[1]	*	+	-	-	0.00
	LR22	0.01	NS[1]	*	+	- 3.80	16.0	5.56
	LR23	0.01	NS[1]	*	+	- 3.00	64.2	30.52
	CP 1	0.01	30.00	*	+	- 1.56	2.6	55.82
	R 1	0.04	24.47		+	-53.10	46.37	30.12

[1]NS - sample lost

[2]ND - not determined

Generally the success of hatching or survival can be increased under laboratory conditions, assuming conditions are optimal. Culture conditions for this study were assumed optimal. Granted, the use of low salinity water in June and July may have reduced hatching success to some degree, but higher salinity water was used in August and per cent hatch was still low, even for crabs collected at the two seaside stations.

Only two studies have been found which report quantitative data on blue crab egg hatchability. Robertson (1938) obtained a maximum of 20 per cent hatch when eggs were hatched in a "grit cloth cage" suspended from a float in Lynnhaven Bay. Only 2 per cent hatch was achieved in laboratory watch glasses. Sandoz and Rogers (1944) obtained 80-90 per cent hatch in the laboratory using water of 23 to 28 °/oo. Judging from the results of the latter study, the values of per cent hatch reported herein are very low.

Roberts (VIMS, personal communication, 1979) conducted some simple tests to determine the effect of air exposure on blue crab egg hatchability and survival of the stage I zoeae. His results indicated that air exposure decreases the success of hatching and larval survival. Review of field and laboratory procedures for ovigerous crabs used in the present study suggests three possible causes for low per cent hatch. First, reduced hatching success could have resulted from air exposure after capture. Five to fifteen minutes elapsed between removal from the crab traps and placement in appropriately coded containers. The time varied as a function of the number of

crabs captured and the weather (high winds plus rough seas slowed the process). Second, and most important, ovigerous crabs and their eggs were kept moist in a small quantity of water contained within a plastic tray. As evidenced by the dark yellow color of the water in the plastic trays, the water became fouled by the crabs' excretory products (which contains metabolic by-products, mainly high levels of ammonia (Kaestner, 1970), the toxicity of which has not been determined for blue crab eggs). Third, crabs were removed from the fouled water in the laboratory and were left out of water for five minutes while they were measured and eggs were dissected from the abdomen.

In August, 1978, an attempt was made to determine the effect, on eggs, of transportation in water fouled by the females' excretory products. Approximately 200 eggs from each of the three crabs collected at Bradford Bay (CP) on the Eastern Shore were removed and incubated in the field and 200 more eggs were removed and incubated upon arrival at the laboratory. The results for the hatchability experiment and the 95% confidence interval for per cent hatch (Diem, 1962) are presented in Table 4. Hatching success was obviously reduced when eggs were transported attached to the female in a small quantity of water. Originally, the study proposed to remove and incubate the eggs at the time of collection, but the necessary boat for the work was not available.

Some eggs may have been adversely affected by the low salinity culture water used during June and July. By comparing the hatching

TABLE 4

95 per cent confidence interval on per cent hatch for ovigerous crabs collected in Bradford Bay, Eastern Shore. A - field-incubated eggs, B - eggs incubated on shore (see text).

Crab	% Hatch*	(95% confidence interval)
CP 1 A	93.27	89.81 - 95.83
1 B	55.82	50.38 - 62.04
CP 2 A	91.58	87.85 - 94.45
2 B	85.04	81.15 - 88.39
CP 3 A	98.68	96.84 - 99.43
3 B	82.31	76.81 - 86.44

*Per cent hatch values were not transformed for this analysis.

success for the three months, it appears that any reduction in hatching, resulting from growth in low salinity water, was minimal. The dramatic increase in per cent hatch in July over June was the result of a change in methodology. In August when only high salinity water was used, the overall mean and median per cent hatch decreased slightly.

It is interesting that eggs of crabs collected from Lynnhaven Roads (part of a state-designated crab sanctuary) during June and August exhibited poor hatching success (refer back to Table 3).

It is no wonder that a meaningful relationship was not observed between egg Kepone concentration and egg hatchability. Even if an effect on hatchability from Kepone in eggs had been present, it would have been overshadowed by other factors which reduced hatchability to a greater extent. Conversely, the concentrations of Kepone found in the blue crabs in the present study may not have an effect on hatchability, a possibility which can not be verified at present.

In conclusion, no relationship between egg Kepone concentration and the success of blue crab eclosion could be established from the data. In addition to the culture methodology, poor hatching success could also be attributed to the past history or health of the adult crab.

Larval Survival and Egg Kepone

The two most obvious features of the larval studies are the high incidence of prezoeae and the overall low survival of larvae. With

the exception of an ovigerous female collected at York Spit in August, all crabs at each station during all three sampling months yielded prezoeae. Prezoeae are characterized by being enveloped in an embryonic cuticle. The dorsal and lateral spines are folded forward under the cuticle and are scarcely visible (Churchill, 1942). The rostrum fits snugly on the ventral side. It too is very insconspicuous (Churchill, 1942). Results from the present study and others (Churchill, 1942; Robertson, 1938; Sandoz and Rogers, 1944) showed that prezoeae do not swim but remain on the bottom of the culture vessel. Robertson (1938) and Churchill (1942) reported that the prezoeal stage, after several hours, sheds the embryonic cuticle and emerges as a normal stage I zoeae. Out of several thousand prezoeae, Sandoz and Rogers (1944) never observed any to shed the embryonic cuticle and become a normal stage I zoeae. Costlow and Bookhout (1959) never observed the prezoeae in their larval studies.

The production of prezoeae usually stems from unfavorable environmental conditions. Sandoz and Rogers (1944) attributed prezoeae production to unfavorable salinity conditions and infection by bacteria or fungi. Roberts (1970) stated that the prezoeae for Pagurus longicarpus is not a "true planktonic stage" and its appearance stemmed from suboptimal culture conditions. Porter (1960) suggested that Menippe mercenaria prezoeae may not be normal planktonic stages. They were most often observed when the "viability of hatching zoeae was poor."

The reasons for prezoeae production in the present study appears to have stemmed from the transport procedures used for the ovigerous female crab. To determine whether prezoeae were produced as a result of the transport procedures used for the ovigerous female, a simple comparison was made using the ovigerous crabs collected in August. The quantity of prezoeae hatched from field-incubated eggs was compared to that from crabs which were transported for several hours in a small quantity of water (Table 5). In every instance, fewer prezoeae hatched from field-incubated eggs than from those eggs incubated several hours later.

Costlow and Bookhout (1959) obtained 5.4% survival to the first crab in 26.7 °/oo seawater. Rust and Carlson (1960) were only able to maintain blue crab larvae for 24 days, and none survived to the second zoeae. Sulkin has reported the results from several blue crab larval development studies, the latest of which (1978) reports 53% survival (determined from the data presented in Table II of Sulkin (1978) to the megalopa when larvae were fed polychaete larvae (Hydroides dianthus) and Artemia nauplii.

The very low larval survival rate obtained in the present study is very pronounced when compared to the data cited above. Furthermore, it is striking to note that only two larvae out of more than 4000 survived to the megalopal stage (none metamorphosed) and Kepone apparently had no effect on larval developmental parameters (i.e., survival, instar duration, and external morphology). Whether

TABLE 5

95 per cent confidence interval on per cent hatch as prezoeae for ovigerous crabs collected in Bradford Bay, Eastern Shore. A - field-incubated eggs, B - eggs incubated on shore (see text).

Crab	% Prezoeae*	(95 % confidence interval)
CP 1 A	0.00	(0.00 - 1.22)
1 B	2.56	(1.02 - 8.77)
CP 2 A	3.07	(1.42 - 5.73)
2 B	11.80	(8.82 - 15.38)
CP 3 A	2.34	(0.94 - 4.78)
3 B	12.62	(8.77 - 17.40)

*Per cent hatch values were not transformed for this analysis.

an effect is masked or not is altogether unknown at this point and can not be resolved with the present data.

Several possible explanations for poor larval survival come to mind: 1) reduced survival resulting from an unidentified pollutant and/or environmental variables, 2) reduced survival resulting from culture technique, 3) reduced survival resulting from handling procedures used for the ovigerous female crab, and 4) reduced survival resulting from inadequate nutrition.

1) The reduced survival rate which was observed may have stemmed from an unidentified pollutant and/or environmental variables. There is no way to document these effects from the present data since the past history of the crab is unknown.

2) One might suspect that the use of low salinity larval culture water in June and July resulted in the observed low larval survival rate. The effects from this were probably trivial since survival was still exceptionally low (as compared to the studies cited earlier) in August when the prescribed culture water was used throughout.

3) If poor hatching success resulted from the handling procedures for the ovigerous female, then the effect may persist during larval development. To test this, the survival of larvae from Bradford Bay, Eastern Shore, was examined (Table 6). Field-incubated eggs from two of the three crabs hatched larvae with a higher survival rate of zoeae I than eggs incubated several hours later (survival of the zoeae II was too low to treat the data as above. The trend for

TABLE 6

95 per cent confidence interval on per cent survival of stage I zoeae for crabs collected in Bradford Bay, Eastern Shore. A - field-incubated eggs, B - eggs incubated on shore (see text).

Crab	% Survival*	(95 % confidence interval)
CP 1 A	8.92	(2.92 - 19.46)
1 B	10.00	(3.77 - 19.72)
CP 2 A	53.33	(40.00 - 66.33)
2 B	43.30	(30.37 - 56.99)
CP 3 A	41.67	(29.07 - 55.12)
3 B	2.94	(0.34 - 10.46)

*Per cent survival values for stage I zoeae were not transformed for this analysis.

greater survival of larvae from field-incubated eggs is not very strong since the 95 per cent confidence interval overlaps for one of the two crabs.

4) If the larvae were not receiving an essential nutrient in their diet then low survival rate may result. Sulkin (1978) has addressed the question concerning the 'best' food for blue crab larval survival, but the diet of the food organism has not been addressed in any papers reviewed with respect to blue crab larval development. Roberts (VIMS, personal communication, 1979) obtained 90% survival of the stage I zoeae when reared on a diet of rotifers (Brachionus plicatilus) fed the flagellated unicelluar algae, Tetraselmis suecica. Sulkin (1978) used Dunaliella sp.-fed rotifers in conjunction with polychaete larvae and Artemia nauplii as food for blue crab larvae and obtained 63% survival to the megalopa. In the present study, the same rotifer (different strain) was used by Chlorella sp., a non-motile unicellular alga, was used as food. It is possible that Chlorella-fed rotifers are not as nutritionally valuable for blue crab larvae as Testraselmis- or Dunaliella-fed rotifers. The biochemical composition of algae can be very different, even within genera (Stewart, 1974), thus it is conceivable that poor larval survival stems from poor nutrition.

In general, decapods lack the ability to synthesize sterols (fused carbon-ring compounds generally used in lipid metabolism) and these compounds must be obtained by way of the diet (Dadd, 1970; Teshima and Kanazawa, 1971). In particular, certain decapod larvae

have been shown incapable of synthesizing sterols from precursors (Whitney, 1969). It seems reasonable to suggest that the diet fed to blue crab larvae in the present study may have lacked a necessary component. Furthermore, Sulkin (1975) has suggested that a lipid component is required in late instar blue crab larvae if they are to complete metamorphosis. Diets that were successful in promoting development were larval invertebrates derived from telolecithal and centrolecithal eggs.

Although there was no indication that Kepone in blue crabs eggs had a detrimental effect on hatchability or larval survival, significant amounts of Kepone in female crabs are passed out of the crab in the eggs during spawning (Roberts and Leggett, 1980). Thus apparently normal larvae may develop, containing various concentrations of Kepone, and may in turn be consumed by second level predators. Continued bioconcentration in higher levels of the food web may result in highly contaminated organisms which, with respect to fishery items, will be unfit for human consumption.

To summarize, the frequent occurrence of prezoeae and the low larval survival rate observed in the present study appears to have stemmed from preculture handling procedures used for the ovigerous female crab. It is also suggested that the larvae may have been malnourished. Judging from Table 6 and past studies of blue crab larval development, preculture handling of the ovigerous female crabs does not seem to fully explain the low larval survival rate observed. The major cause of low survival rate is not clear at this time.

CONCLUSIONS

1. Levels of egg Kepone in blue crabs (Callinectes sapidus) up to 1.45 µg/g had no demonstrable effect on embryogenesis, egg hatchability, or larval survival.

2. Levels of egg Kepone up to 1.45 µg/g caused no larval abnormalities.

Appendix A. Blue crab egg kepone data and hatchability data for the months of June, July, and August. NH - no hatch, ND - not determined, and NS - no sample. Kepone concentrations are given as µg/g.

Data for the month of June

Crab	Egg Kepone	Days to First Hatch	Total Days Culture	% Hatch$_{(I)}$	$\sin^{-1}$ % Hatch$^{1/2}_{(I)}$	% Hatch$_{(A)}$	$\sin^{-1}$ % Hatch$^{1/2}_{(A)}$	$\sin^{-1}$ % Eggs Lost/Gained$^{1/2}$
HB 1	0.08	5	6	44.29	41.72	50.67	45.38	-20.79
2	0.45	4	5	45.81	42.60	46.38	42.92	- 6.29
4	0.03	5	6	42.11	40.46	28.54	32.29	23.42
5	0.09	5	6	48.69	44.25	63.05	52.57	-28.52
6	0.56	3	4	58.86	50.10	68.00	55.54	-21.47
7	0.02	6	7	72.81	58.57	83.70	66.19	-21.13
8	0.01	5	6	51.09	45.63	49.48	44.76	10.14
9	0.03	3	4	60.43	51.02	65.27	53.89	-16.54
HS 1	0.45	7	8	48.89	44.36	41.64	40.19	24.65
2	0.11	7	8	60.62	51.13	65.61	54.10	-16.00
3	0.19	7	8	27.25	31.47	29.48	32.88	-15.89
4	0.22	NH	19	00.00	-	00.00	-	-
5	1.45	5	6	21.21	27.42	20.39	26.84	-11.54
6	0.03	6	7	37.90	38.00	49.12	44.50	-28.52
7	0.18	8	9	54.99	47.87	67.09	54.99	-25.10
8	0.05	7	8	8.86	17.32	18.00	25.07	-17.36
9	0.02	6	7	70.25	56.94	66.00	54.31	- 2.56
LR 1	0.02	8	9	27.42	31.58	32.00	34.43	-22.14
2	0.02	8	9	40.10	39.29	43.90	41.50	-17.36
3	0.05	8	9	45.58	42.47	81.67	64.65	-41.67
4	0.01	8	9	27.53	31.65	38.42	38.31	-31.82
5	0.53	7	8	34.65	36.06	53.64	47.09	-36.51
6	0.53	7	8	34.65	36.06	53.64	47.09	-36.51
7	0.77	8	9	67.03	54.96	81.58	64.58	-24.96

Data for the month of June (concluded)

Crab	Egg Kepone	Days to First Hatch	Total Days Culture	% $Hatch_{(I)}$	Sin^{-1} % $Hatch^{1/2}_{(I)}$	% $Hatch_{(A)}$	Sin^{-1} % $Hatch^{1/2}_{(A)}$	Sin^{-1} % Eggs $Lost/Gained^{1/2}$
PP 1	0.02	6	7	91.63	73.19	85.50	67.61	-12.11
2	0.68	5	6	32.93	35.02	40.00	39.23	-17.66
YS 2	0.02	6	7	91.64	73.19	85.50	67.61	15.57
3	0.02	7	8	76.76	61.18	76.29	60.86	4.44

Data for the month of July

Crab	Egg Kepone	Days to First Hatch	Total Days Culture	% $Hatch_{(I)}$	Sin^{-1} % $Hatch^{1/2}_{(I)}$	% $Hatch_{(A)}$	Sin^{-1} % $Hatch^{1/2}_{(A)}$	Sin^{-1} % Eggs $Lost/Gained^{1/2}$
HB 15	0.49	3	5	99.76	87.19	97.45	80.81	8.91
16	0.78	7	9	56.31	48.63	74.54	59.69	-29.07
17	0.16	5	7	91.19	72.73	92.52	74.12	- 6.80
18	0.41	7	9	39.49	38.93	ND	-	-
19	0.34	NH	6	00.00	-	00.00	-	-
20	0.49	6	8	68.72	55.99	89.23	70.84	-28.66
21	1.16	7	9	60.50	51.06	73.33	58.90	-24.73
HS 10	0.01	7	10	60.47	51.04	64.91	53.67	-15.12
11	0.28	3	6	77.69	61.81	85.90	67.95	-18.05
12	1.26	4	6	94.01	75.83	95.50	77.76	- 7.27
13	0.01	2	6	96.62	79.41	78.74	62.54	28.45
14	0.38	5	9	71.30	57.60	76.43	60.95	-15.00
15	0.57	1	5	88.85	70.49	90.31	71.86	- 7.27
16	0.57	4	7	42.61	40.75	85.00	67.21	-44.94
LR 8	0.03	1	5	83.96	66.39	89.13	70.75	-13.94
9	0.01	1	5	73.62	59.10	68.77	56.02	15.45
PP 13	0.59	6	8	59.18	50.29	62.53	52.26	-13.44
14	0.18	6	9	26.68	31.09	31.48	34.13	-23.03
15	0.34	5	9	59.60	50.53	65.12	53.80	-16.95
WT 3	0.20	4	8	91.89	73.46	98.71	83.48	-15.23
4	0.01	1	5	23.05	28.69	21.11	27.35	17.66

Data for the month of July (concluded)

Crab		Egg Kepone	Days to First Hatch	Total Days Culture	% Hatch$_{(I)}$	Sin^{-1} % Hatch$^{1/2}_{(I)}$	% Hatch$_{(A)}$	Sin^{-1} % Hatch$^{1/2}_{(A)}$	Sin^{-1} % Eggs Lost/Gained$^{1/2}$
YS	4	0.01	6	8	96.13	78.65	93.17	74.86	10.31
	5	0.01	7	14	10.57	19.00	ND	-	-
	6	0.01	6	9	48.59	44.19	49.71	44.83	- 8.72

Data for the month of August

Crab	Egg Kepone	Days to First Hatch	Total Days Culture	% $Hatch_{(I)}$	Sin^{-1} % $Hatch^{1/2}_{(I)}$	% $Hatch_{(A)}$	Sin^{-1} % $Hatch^{1/2}_{(A)}$	Sin^{-1} % Eggs $Lost/Gained^{1/2}$
HB 31	0.18	6	9	77.46	61.65	76.34	60.90	- 6.93
32	0.12	5	7	67.07	54.98	98.24	82.37	-34.27
33	0.18	3	5	77.45	61.65	97.13	80.25	-26.78
34	0.29	6	7	89.78	71.35	99.51	85.97	-18.24
HS 23	0.01	5	7	68.91	56.11	67.01	54.95	- 9.68
24	0.01	3	5	74.29	59.53	ND	-	-
25	0.01	3	4	79.56	63.12	87.32	69.14	-17.36
26	0.03	3	4	74.45	59.64	98.55	83.09	-21.89
27	0.01	3	4	79.37	63.00	86.34	68.31	-18.72
LR 15	0.01	8	9	80.60	63.87	97.74	81.35	-24.73
16	0.01	1	3	55.78	48.32	37.50	37.76	44.26
17	0.03	2	3	21.88	27.89	23.43	28.95	-14.89
18	0.05	NH	1	00.00	-	00.00	-	-
19	NS	7	7	1.28	6.50	ND	-	-
20	0.04	NH	2	00.00	-	00.00	-	-
21	0.01	NH	3	00.00	-	00.00	-	-
22	0.01	6	13	16.00	23.56	16.88	24.26	-11.24
23	0.01	8	10	64.16	67.93	85.89	67.93	- 9.97
WT 15	0.40	4	6	99.00	84.13	98.96	84.13	00.00
YS 19	0.03	5	11	2.66	9.38	2.18	9.69	-14.42
CP 1	0.01	6	10	55.82	48.34	58.16	49.70	- 1.56
2	0.01	4	6	85.04	64.25	93.85	75.64	-15.36
3	0.01	3	6	82.31	65.13	93.04	74.71	-19.86

Data for the month of August (concluded)

Crab		Egg Kepone	Days to First Hatch	Total Days Culture	% $Hatch_{(I)}$	Sin^{-1} % $Hatch^{1/2}_{(I)}$	% $Hatch_{(A)}$	Sin^{-1} % $Hatch^{1/2}_{(A)}$	Sin^{-1} % Eggs $Lost/Gained^{1/2}$
R	1	0.04	7	9	46.37	42.92	69.05	56.20	-46.78
	2	0.01	4	6	88.37	70.06	99.13	84.65	-21.30
	3	0.08	6	8	65.66	54.13	98.31	82.52	-35.18
	4	0.01	6	9	31.38	34.07	36.11	36.94	-21.22
	5	0.01	7	8	8.55	17.00	8.19	16.63	12.11

Appendix B. Egg Kepone, prezoeae (PreZ), and zoeae I and II data for the months of June, July, and August. NH - no hatch, NSI - no stage I photopositive larvae were obtained, ND - not determined, NS - no sample, BL - bowl lost. Kepone concentrations are given as μg/g.

Data for the month of June

Crab	Kepone	Zoeae I			Zoeae II		Mean Time to II	Max. # Instars	Mean Time Death
		$\sin^{-1}$ % $PreZ^{1/2}$	% Survival	$\sin^{-1}$ % $Survival^{1/2}$	% Survival	$\sin^{-1}$ % $Survival^{1/2}$			
HB 1	0.08	00.00	1.67	7.42	00.00	00.00	7.0	1	5.5
2	0.45	few	15.00	22.79	00.00	00.00	7.2	1	6.9
4	0.03	00.00	00.00	00.00				0	5.8
5	0.09	32.60	00.00	00.00				0	1.7
6	0.56	16.19	1.67	7.42	00.00	00.00	9.2	1	4.5
7	0.02	00.00	1.67	7.42	00.00	00.00	9.0	1	7.0
8	0.01	00.00	15.00	22.79	00.00	00.00	6.5	1	4.9
9	0.03	3.58	1.67	7.42	00.00	00.00	6.8	1	8.7
HS 1	0.45	53.36	1.67	7.42	00.00	00.00	7.0	1	5.4
2	0.11	00.00	1.67	7.42			5.0	1	8.0
3	0.19	66.31	00.00	00.00				0	2.6
4	0.22	NH	-	-				-	-
5	1.45	00.00	13.33	21.42			7.4	2	6.5
6	0.03	00.00	00.00	00.00				0	6.6
7	0.18	00.00	00.00	00.00				0	9.3
8	0.05	43.54	00.00	00.00				0	2.7
9	0.02	00.00	00.00	00.00				0	6.6
LR 1	0.02	00.00	00.00	00.00				0	4.7
2	0.07	00.00	5.00	12.92	00.00	00.00	5.7	1	5.8
3	0.05	00.00	3.33	10.52	00.00	00.00	8.5	1	7.3
4	0.01	00.00	00.00	00.00				0	3.7
5	0.53	00.00	1.67	7.42	00.00	00.00	5.0	1	3.7
6	0.29	90.00	NSI	-	-	-	-	-	-
7	0.77	00.00	1.67	7.42	1.67	7.42	8.0	2	6.7

Data for the month of June (concluded)

Crab	Kepone	Zoeae I			Zoeae II				
		% PreZ	$\sin^{-1}$ % PreZ$^{1/2}$	% Survival	$\sin^{-1}$ % Survival$^{1/2}$	% Survival	$\sin^{-1}$ % Survival$^{1/2}$	Mean Time to II	Max. # Instars	Mean Time Death
PP 1	0.14	few		00.00	00.00				0	6.6
2	0.68	ND		00.00	00.00				0	5.9
YS 2	0.02	14.69		00.00	00.00				1	7.4
3	0.02	few		2.50	9.10	00.00	00.00	7.0	1	7.1

Data for the month of July

Crab	Kepone	Zoeae I			Zoeae II		Mean Time to II	Max. # Instars	Mean Time Death
		$\sin^{-1}$ % $PreZ^{1/2}$	% Survival	$\sin^{-1}$ % $Survival^{1/2}$	% Survival	$\sin^{-1}$ % $Survival^{1/2}$			
HB 15	0.49	14.12	6.67	14.96	00.00	00.00	9.0	1	2.5
16	0.78	38.94	27.50	31.63	00.00	00.00	8.5	1	7.5
17	0.16	9.04	39.25	38.79	1.67	7.48	7.9	2	9.4
18	0.41	37.14	00.00	00.00				0	4.2
19	0.34	NH	NH	-				-	-
20	0.49	53.83	8.33	16.78	00.00	00.00	8.0	1	8.1
21	1.16	35.35	00.00	00.00				0	4.0
HS 10	0.01	46.93	00.00	00.00				0	8.1
11	0.28	20.54	7.02	15.36	1.75	7.61	9.0	3	5.0
12	1.26	29.03	55.00	47.87	5.00	12.92	9.9	2	12.6
13	0.01	9.10	23.33	28.88	5.00	12.92	8.9	3	10.1
14	0.38	39.76	BL	-				-	-
15	0.57	8.97	00.00	00.00				0	8.2
16	0.57	30.58	33.75	35.52	7.50	15.89	8.4	2	7.3
LR 8	0.03	53.41	1.67	7.42	00.00	00.00	9.0	1	5.6
9	0.01	90.00	NSI	-				-	-
PP 13	0.59	34.39	26.74	31.14	00.00	00.00	7.1	1	7.8
14	0.18	49.80	NSI	-				-	-
15	0.34	18.54	46.67	43.09	1.67	7.42	8.6	6	19.1
WT 3	0.20	32.22	00.00	00.00				0	4.6
4	0.01	59.04	00.00	00.00				0	4.7

Data for the month of July (concluded)

Crab		Kepone	Zoeae I			Zoeae II				
			% PreZ	$\sin^{-1}$ % PreZ$^{1/2}$	% Survival	$\sin^{-1}$ % Survival$^{1/2}$	% Survival	$\sin^{-1}$ % Survival$^{1/2}$	Mean Time to II	Max. # Instars	Mean Time Death
YS	4	0.01		33.24	00.00	00.00				0	6.7
	5	0.01		00.00	00.00	00.00				0	5.3
	6	0.01		33.72	1.67	7.42	1.67	7.42	14.0	3	6.2

Data for the month of August

Crab	Kepone	Zoeae I			Zoeae II		Mean Time to II	Max. # Instars	Mean Time Death
		Sin^{-1} % $PreZ^{1/2}$	% Survival	Sin^{-1} % $Survival^{1/2}$	% Survival	Sin^{-1} % $Survival^{1/2}$			
HB 31	0.18	27.04	8.73	17.23	00.00	00.00	7.8	1	7.8
32	0.12	26.82	65.00	53.73	1.67	7.42	6.2	2	11.4
33	0.18	12.10	60.11	50.83	13.33	21.42	6.0	5	13.3
34	0.29	24.10	71.13	57.50	1.67	7.42	7.6	2	11.1
HS 23	0.01	61.64	42.50	40.69	00.00	00.00	5.9	1	9.4
24	0.01	54.25	38.33	38.25	1.67	7.42	6.9	2	10.5
25	0.01	47.40	46.67	43.09	21.67	27.74	6.9	3	9.9
26	0.03	00.00	60.09	50.82	5.09	13.04	7.6	2	10.6
27	0.01	10.61	54.41	47.53	17.46	24.70	6.1	6	12.5
LR 15	0.01	33.56	56.67	48.83	5.00	12.92	7.4	5	11.8
16	0.01	90.00	NSI	-				-	-
17	0.03	76.61	10.00	18.44	00.00	00.00	9.0	1	15.0
18	0.05	NH	NH	-				-	-
19	NS	00.00	NSI	-				-	-
20	0.04	NH	NH	-				-	-
21	0.01	NH	NH	-				-	-
22	0.01	13.64	59.90	50.71	00.00	00.00	7.5	1	9.2
23	0.01	33.54	33.51	35.37	00.00	00.00	6.5	1	9.5
WT 15	0.4	21.16	55.00	47.87	13.33	21.42	8.3	3	11.9
YS 19	0.03	00.00	18.75	25.66	2.08	8.29	7.6	2	5.3

Data for the month of August (concluded)

Crab		Kepone	Zoeae I		Zoeae II		Mean Time to II	Max. # Instars	Mean Time Death
			$\sin^{-1}$ % $PreZ^{1/2}$	% Survival	$\sin^{-1}$ % $Survival^{1/2}$	% Survival	$\sin^{-1}$ % $Survival^{1/2}$		
CP	1	0.01	9.21	10.00	18.43	00.00	00.00	8.0	1	8.5
	2	0.01	20.09	43.33	41.17	8.70	22.03	6.0	5	10.6
	3	0.01	20.81	2.94	9.87	00.00	00.00	8.8	1	6.5
R	1	0.40	33.29	30.43	33.48	00.00	00.00	7.6	1	9.6
	2	0.01	7.60	67.08	54.99	1.67	7.42	8.3	2	9.5
	3	0.08	21.80	12.55	20.75	00.00	00.00	7.9	1	8.4
	4	0.01	16.01	5.00	12.92	00.00	00.00	9.0	1	7.5
	5	0.01	00.00	19.07	25.90	00.00	00.00	7.5	1	8.3

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