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Effects of thermal shock on larvae of the oyster, Crassostrea virginica (Gmelin)

Robert J. Diaz
College of William and Mary - Virginia Institute of Marine Science

EFFECTS OF THERMAL SHOCK ON LARVAE OF THE OYSTER, CRASSOSTREA VIRGINICA (GMELIN)

Robert James Diaz
Gloucester Point, Virginia

B.A., LaSalle College, 1968

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Author

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Morris Brehmer, Ph.D.

Michael Bender, Ph.D.

Dale Calder.
Dale Calder, Ph.D.

TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

ABSTRACT

Larvae of the American oyster Crassostrea virginica (Gmelin) were subjected to thermal shocks of varying magnitude at different larval ages. The effects were assessed in terms of mortality, growth and setting success. Growth of larvae acclimated at 25 C and 20 ‰ salinity was not affected by a thermal shock of 10 C or 15 C, but a 20 C thermal shock had a detrimental effect on growth. Mortality of oyster larvae increased significantly following thermal shocks of 10, 15, and 20 C, and was higher for older than for younger larvae. Setting rates of the control and 10 C shocked larvae were not significantly different, but larvae receiving 15 or 20 C shocks had significantly lower setting success. Growth rate and setting also seemed to be affected by the age at which larvae received a thermal shock.

EFFECTS OF THERMAL SHOCK ON LARVAE OF THE OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN)

INTRODUCTION

A thermal shock may be defined as a sudden rise in temperature over a very short time interval and a subsequent return to ambient temperature over a longer period of time. The probability of an estuarine organism encountering a thermal shock during its lifetime is increasing due to the increasing number of nuclear and fossil fueled steam electric stations locating on tidal systems.

The demand for electricity will more than double by the year 1980, increasing by approximately 657 billion kilowatt hours, with only 4% of the electricity generated by non-thermal enriching means (Southern Governors Task Force, 1970). Approximately 5,500 BTU of waste heat are carried to the aquatic environment by cooling water for every kilowatt hour of electricity generated. Organisms entrained in the cooling water pumped into the electric plants receive a thermal shock as the water passes through condensers. It is essential to determine the tolerance of different species to the sudden temperature rises that they may encounter in their environment.

Newell (1966) indicated that short term fluctuations in temperature produce fluctuations in the metabolism of poikilotherms, based on activity and oxygen consumption measurements. Acclimation to such short term environmental changes are impossible because an organism cannot compensate for a thermal shock without an initial overshoot or shock reaction. Acclimation is essentially a long term

adjustment during which an organism slowly adjusts to changing environmental conditions.

Davis and Calabrese (1964, 1969) studied the thermal tolerance of larvae of the oyster, Crassostrea virginica (Gmelin). Larvae were subjected to temperatures ranging from 13 to 37.5 C for their entire life, and growth and survival rates were estimated. Davis and Calabrese reported the optimum growth range for C. virginica larvae to be between 25 - 27 C, and that a temperature above 22.5 C was required for growth. Similar work has been carried out with many organisms, including fishes (Brett, 1956; Parvatheswararo, 1967) and copepods (Halcrow, 1963). These works were concerned with the long range effects of temperature and did not consider the reactions of organisms to short term thermal shock. Little has been published on tolerance of oyster larvae to thermal shock. Lutz, Hidu and Drobeck (1970) studied the effects of temperature change on the setting of C. virginica larvae and reported that a thermal shock of 5 C (from 24 to 29 C) administered to larvae of setting size increased the percentage that set. J. P. Roosenberg (personal communication) is investigating thermal shock reactions of oyster larvae by computing LD_{50} values after exposing them to various temperatures for periods from five seconds to three hours.

Reeve and Cosper (1970) assessed the effects of thermal shock on the survival of the copepod Acartia tonsa. Copepods were subjected to thermal shocks ranging from 3 - 14 C above ambient temperatures of 20 - 30 C for periods of one-half to six hours. No single lethal temperature was found for A. tonsa, but death was directly proportional to the duration of exposure at the increased

temperatures.

The purpose of this study was to determine the effects of thermal shock on growth, mortality, and setting success of oyster larvae. Oysters were chosen for the study because of their economic importance, distribution in estuaries, and proven culture methods. This study was funded by the Virginia Electric and Power Company.

MATERIALS AND METHODS

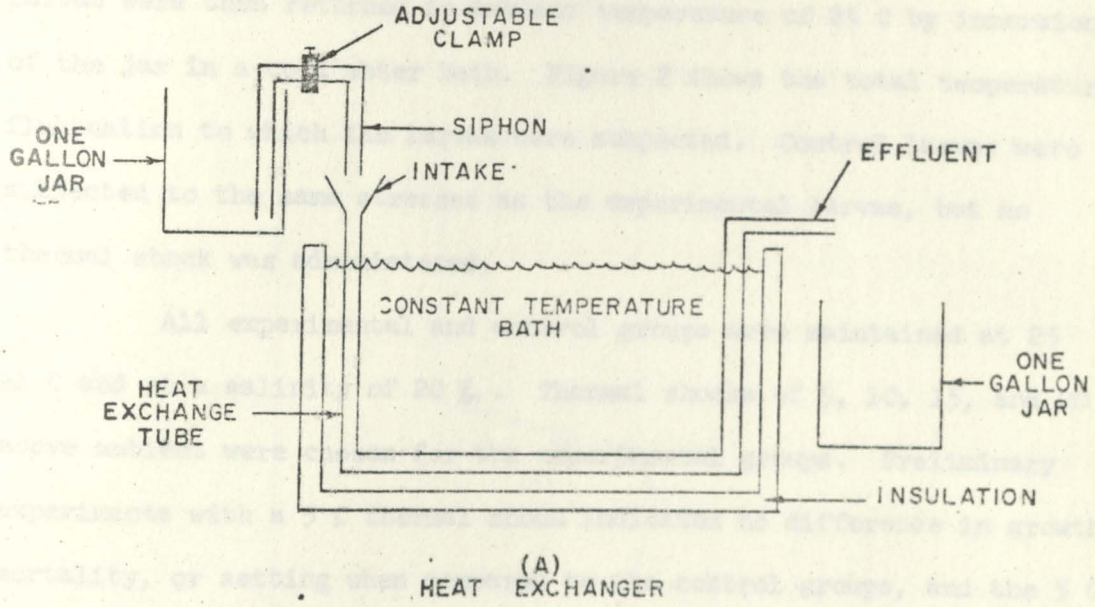
Larvae of the oyster Crassostrea virginica used in this study were obtained from adults spawned in the laboratory at the Virginia Institute of Marine Science. Adults were obtained from the Rappahannock and Potomac rivers. Three different broods were necessary for the completion of all experimental and control runs because the number of larvae that could be maintained under controlled conditions was limited.

Stock cultures of larvae were maintained at 25 C in 80 liter polyethylene containers at a concentration of five to ten larvae per ml. Cultures were fed 20 to 25 ml of pure culture Monochrysis per liter of larval culture every 24 hours. Water in the stock cultures was changed every 48 hours to prevent buildup of metabolic products, dead larvae, and bacteria. Water changing was accomplished by filtering larvae onto stainless steel mesh screens that retained only the larvae. The size of the screen used ranged from 64 μ to 250 μ, depending on the size of the larvae. The polyethylene container was then cleaned and the larvae resuspended in new water filtered through a one micron filter. An antibiotic (0.2 ml/liter of Twin Biotic, Vineland Poultar Co., Vineland, N. J.) was added at each water change to control bacterial populations.

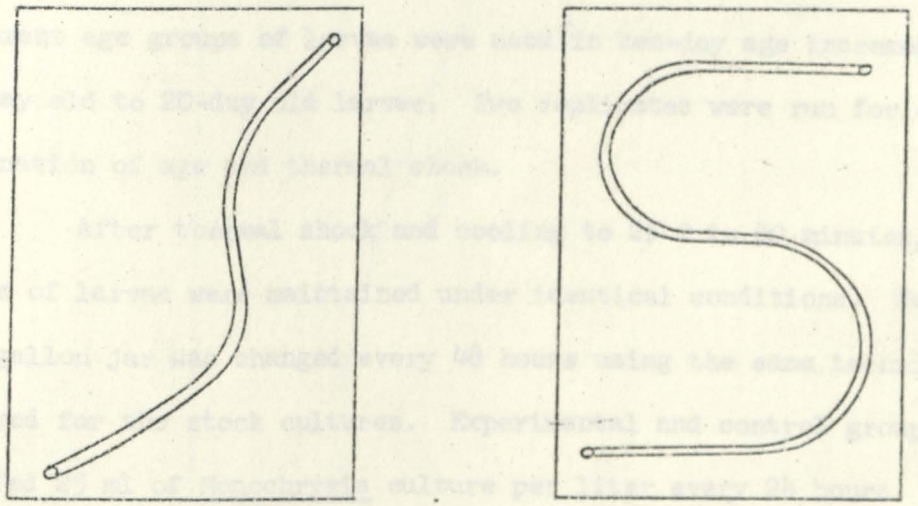
Thermal shocks were administered to the larvae with a heat exchanger (Fig. 1a) consisting of an entrainment tube in a constant temperature bath. A 20-liter stainless steel tank, measuring

40 by 38 cm and surrounded on all four sides and bottom by 5 cm of polystyrene insulation, served as a constant temperature bath. The entire bath was enclosed in a wooden box to prevent deterioration of the insulation and to provide support for the heating units and entrainment tube. The top of the bath was covered with a 6 mm thick plexiglass sheet to reduce evaporation of water used as the heat transfer medium. Heat was provided by a 1000-watt glass immersion heater and two 1000-watt Portatemp heaters. Heated water was circulated by the propeller stirrer and pump on each of the Portatemp units. The three heaters were capable of maintaining temperatures from room temperature to 99 C. Entrainment tubes were made of 25 mm inside diameter borosilicate glass tubing. Larvae passing through the tube received a thermal shock proportional to the temperature of the bath and the flow rate through the tube. Two shapes were developed for the entrainment tubes (Fig. 1b and 1c). One tube was sigmoid, 75 cm long, and provided short entrainment times. The second tube was S-shaped, 150 cm long, and provided longer entrainment times with greater thermal shocks.

Once the constant temperature bath was brought to the appropriate temperature and calibrated for a desired thermal shock, larvae were run through the entrainment tube at a concentration of five to ten per ml. The flow of larvae was sustained by a siphoning action with a residence time in the tube of about five seconds. Residence time was controlled by an adjustable hose clamp on the siphon. Mercury thermometers were located at both ends of the entrainment tube to monitor the magnitude of the thermal shock. Once the appropriate thermal shock was obtained within ±0.5 C, oyster larvae were collected



(A)
HEAT EXCHANGER



(B)
SIGMOID

(C)
"S" SHAPED

HEAT EXCHANGE TUBES

Figure 1. Heat exchanger and heat exchange tubes.
A. Cross section of heat exchanger.
B. Sigmoid heat exchange tube.
C. S-shaped heat exchange tube.

in gallon jars. Three to four liters of water containing shocked larvae were placed in each jar and allowed to cool for 30 minutes. Larvae were then returned to ambient temperature of 25 C by immersion of the jar in a cold water bath. Figure 2 shows the total temperature fluctuation to which the larvae were subjected. Control larvae were subjected to the same stresses as the experimental larvae, but no thermal shock was administered.

All experimental and control groups were maintained at 25 ±1 C and at a salinity of 20 ‰. Thermal shocks of 5, 10, 15, and 20 C above ambient were chosen for the experimental groups. Preliminary experiments with a 5 C thermal shock indicated no difference in growth, mortality, or setting when compared to the control groups, and the 5 C thermal shock tests were discontinued. A control group, receiving no thermal shock, was maintained with each set of shocked larvae. Ten different age groups of larvae were used in two-day age increments from two-day old to 20-day old larvae. Two replicates were run for each combination of age and thermal shock.

After thermal shock and cooling to 25 C in 30 minutes, all groups of larvae were maintained under identical conditions. Water in each gallon jar was changed every 48 hours using the same techniques employed for the stock cultures. Experimental and control groups were both fed 25 ml of Monochrysis culture per liter every 24 hours. Salinity was kept constant by addition of distilled water (if the salinity was above 20 ‰) or evaporated sea salts (if the salinity was below 20 ‰). Salinity was monitored with a Beckman RS7-A salinometer. As the number of viable larvae in the experimental and control groups decreased, the concentration of larvae was maintained at five to ten

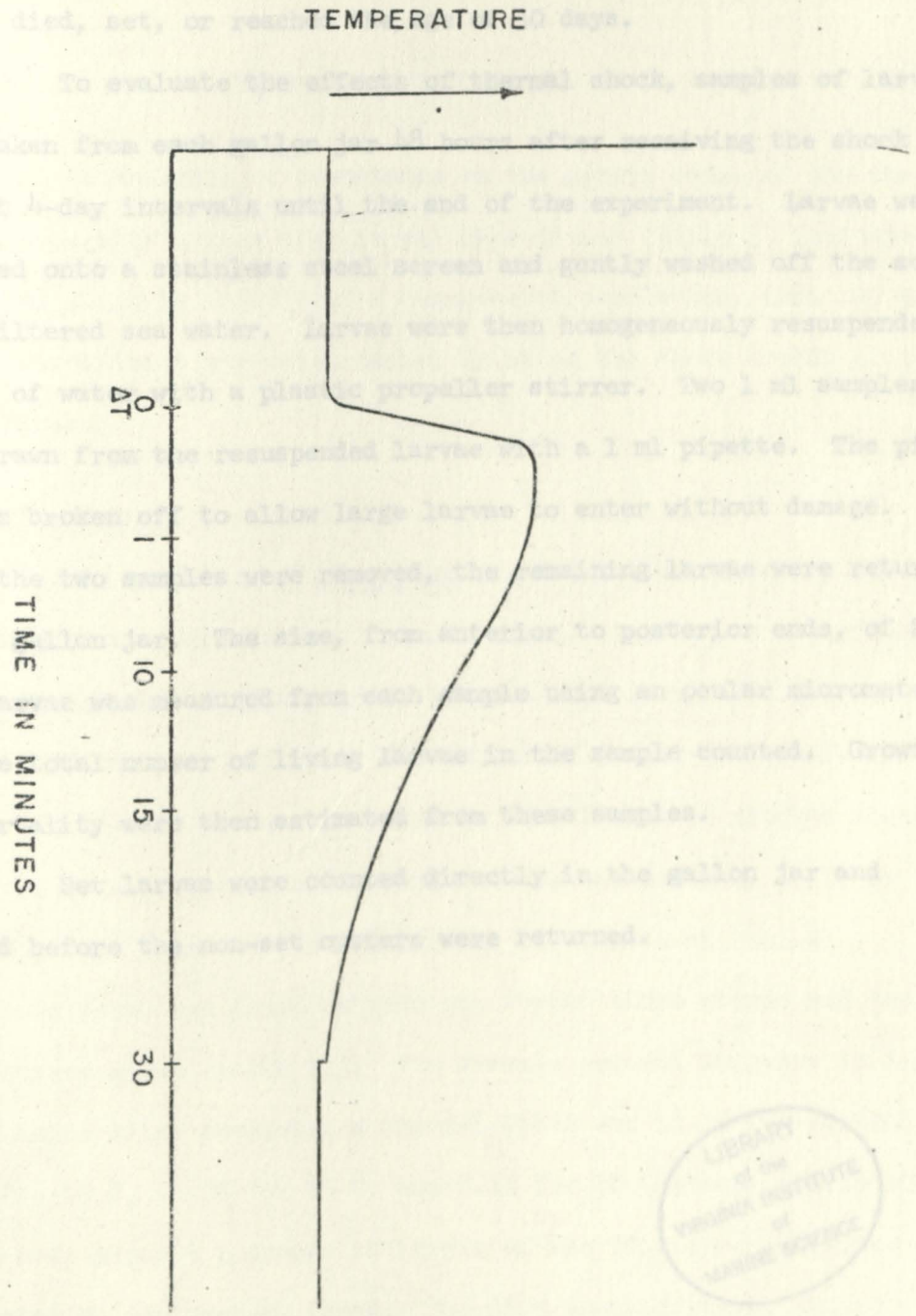


Figure 2. Total temperature fluctuation to which Crassostrea virginica larvae were subjected.

per ml by decreasing the amount of new water added at each water changing. All groups were maintained until all the larvae in a group either died, set, or reached the age of 30 days.

To evaluate the effects of thermal shock, samples of larvae were taken from each gallon jar 48 hours after receiving the shock and then at 4-day intervals until the end of the experiment. Larvae were screened onto a stainless steel screen and gently washed off the screen with filtered sea water. Larvae were then homogeneously resuspended in 300 ml of water with a plastic propeller stirrer. Two 1 ml samples were drawn from the resuspended larvae with a 1 ml pipette. The pipette tip was broken off to allow large larvae to enter without damage. After the two samples were removed, the remaining larvae were returned to the gallon jar. The size, from anterior to posterior ends, of 20 live larvae was measured from each sample using an ocular micrometer, and the total number of living larvae in the sample counted. Growth and mortality were then estimated from these samples.

Set larvae were counted directly in the gallon jar and removed before the non-set oysters were returned.

RESULTS AND DISCUSSION

An analysis of covariance on the growth rates of the three broods of oyster larvae used in all experiments (Table I) indicated that they could be considered a homogeneous population, that is, there was no significant variation caused by using the three broods in the experiments.

Effects on Growth

The growth of C. virginica larvae was influenced by the magnitude of the administered thermal shock. Growth was not affected by thermal shocks of 10 and 15 C, but was impared by a 20 C shock. Analysis of variance and Tukey's-w procedure (Sokal and Rohlf, 1969) run on the 48-hour percent increase in mean length of shocked larvae indicated no significant difference between the control, 10 C, or 15 C shocked groups. However, a significant difference at the .01 confidence level was found between the former three groups and the 20 C shocked group (Table II). The average percent increase in length for 48 hours after receiving a thermal shock was 15.1% for control, 15.8% for 10 C, 12.2% for 15 C, and 5.1% for 20 C groups (Table III). The 48-hour percent increase in length of the 10 C shocked larvae was unaffected by the thermal shock. The 15 C shocked larvae showed some decline in growth and the growth of the 20 C shocked larvae was severely reduced by the thermal shock. At no time did the 48-hour percent increase in length of the 20 C shocked larvae exceed that of

Table I. Analysis of covariance on the three broods of C. virginica larvae used for the thermal shock experiments. Broods were arranged according to thermal shock received.

Analysis of Covariance

Source	X2	SXY	SY2	N-1	SD2	N-2	SD2/N-2
Control group	7066.2	57678.2	528041.7	111	57243.1	110	520.4
10C shocked	7075.4	61197.1	584177.7	111	54868.4	110	498.8
15C shocked	6868.4	57976.4	531578.2	109	42196.2	108	390.7
20C shocked	6494.6	52683.7	486542.2	105	59179.1	104	569.0
Pooled					213486.8	432	494.2
Reg. coef.					1306.7	3	435.6
Common	27504.7	229535.4	2130339.7	436	214793.5	435	493.8
Adj. mean					14732.8	3	4910.9
Total	27523.9	230223.5	2155225.9	439	229526.3	438	

F for Reg. coef. is 0.881. Degrees of freedom are 3.432.

F for Adj. mean is 9.946. Degrees of freedom are 3.435 *

* Significant at the .05 level.

Table II. Analysis of Variance and Tukey's-w procedure for the percent increase in mean length of C. virginica larvae 48 hours after thermal shock.

Analysis of Variance with Arcsine Transformation

Source	Sum of Squares	Df	Mean Square	F ratio
Between groups	802.5	3	267.5	16.634*
Within groups	578.9	36	16.1	
Total	1381.4	39		

*Significant at the .05 level.

Tukey's-w Procedure .01 Level

$$w_{01} = 4.74(S_{\bar{x}})$$

$$S_{\bar{x}} = \frac{s^2}{r} = \frac{16.1}{10} = 1.27$$

$$w_{01} = 6.01$$

	Control	10C	15C	20C	Groups
Means	22.66	23.25	20.06	12.03	Arcsine values
Means	14.89	15.90	11.81	4.32	Percentages

Table III. Percent increase in mean length of C. virginica larvae 48 hours after thermal shock.

Age of larvae when subjected to thermal shock (days)	Control	10C	15C	20C
2	11.0	9.5	11.0	4.4
4	20.2	18.6	16.6	6.7
6	9.6	9.4	4.0	3.2
8	11.0	15.8	14.8	1.8
10	11.2	17.9	12.6	3.5
12	17.2	14.9	10.5	7.7
14	21.0	18.0	16.9	11.8
16	16.4	19.4	14.0	6.8
18	16.4	20.1	15.2	3.8
20	16.6	14.2	6.0	0.1
Mean	15.1	15.8	12.2	5.1

the other three groups and only once did the 15 C shocked larvae exceed the percentage increase of the 10 C shocked group.

Thermal shocks of 15 and 20 C slowed the initial growth of larvae to a greater extent than a 10 C shock. After six to seven days the surviving larvae of all groups grew at approximately the same rate (Figs. 3 - 12). The final size of larvae subjected to a shock at the age of four and six days was greater than the control because the setting of larger control larvae reduced the estimate of their size (Figs. 4, 5). Larvae given a 20 C shock at the age of two and eight days died prematurely, causing the discrepancy in growth within the four groups (Figs. 3, 6). The 14 day old larvae that received a 15 or 20 C shock died prematurely. This premature death was attributed to some lasting effect of thermal shock (Fig. 9).

The age at which larvae received a thermal shock had an effect on growth for at least 48 hours. Control and 10 C shocked groups tended to have greater increases in length per 48 hour interval as the age at which they received the shock increased. The increase of the 15 C shocked group stayed the same as the age at which the larvae were shocked increased. The 20 C shocked group tended to decrease in growth as the age at which the larvae were shocked increased (Figs. 3 - 12).

Effects on Mortality

Mortality of oyster larvae was strongly affected by thermal shock. Analysis of variance and Tukey's-w procedure computed for the 48 hour mortalities of the control, 10, 15, and 20 C shocked groups showed, at the .05 confidence level, that the mortality of each group

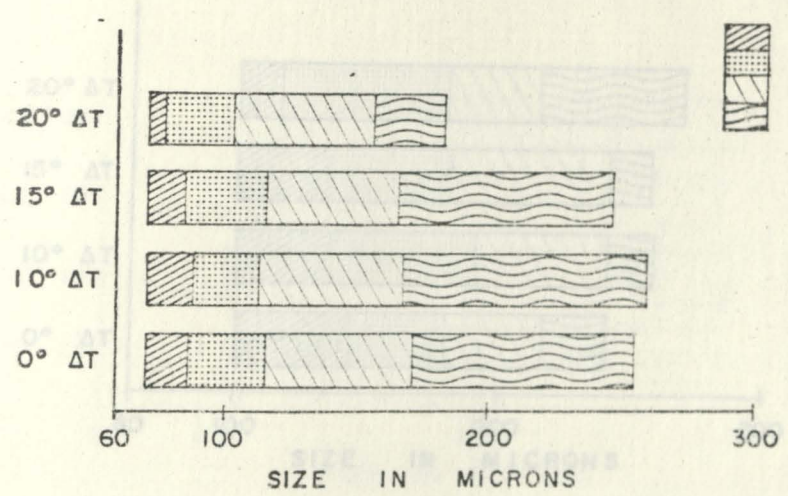


Figure 3. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of two days.

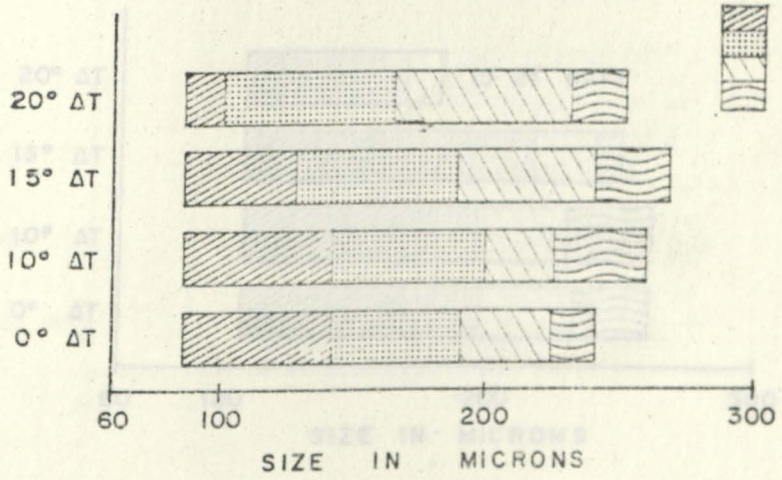


Figure 4. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of four days.

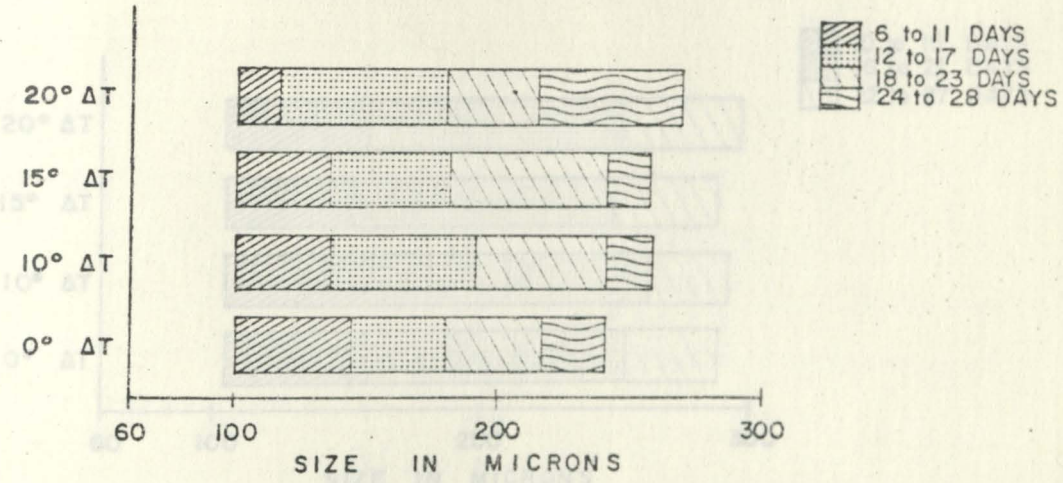


Figure 5. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of six days.

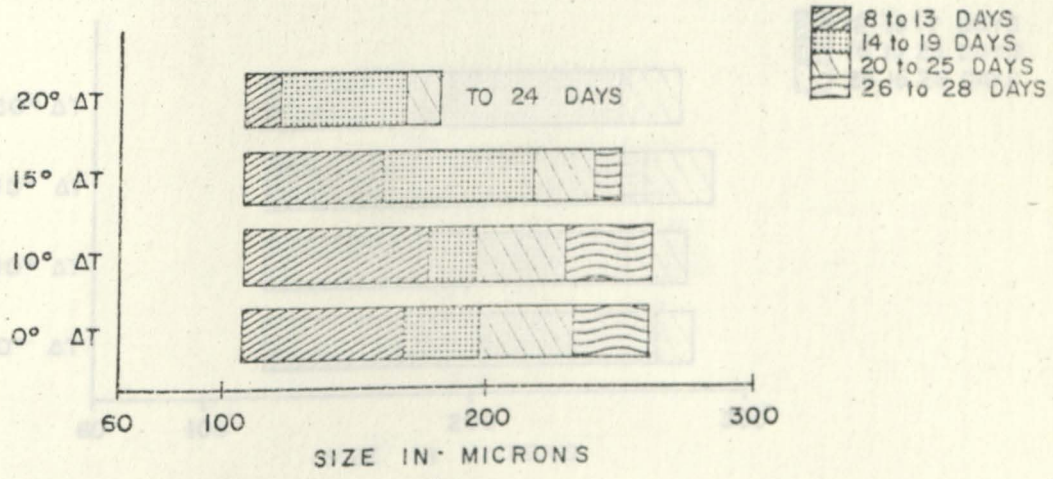


Figure 6. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of eight days.

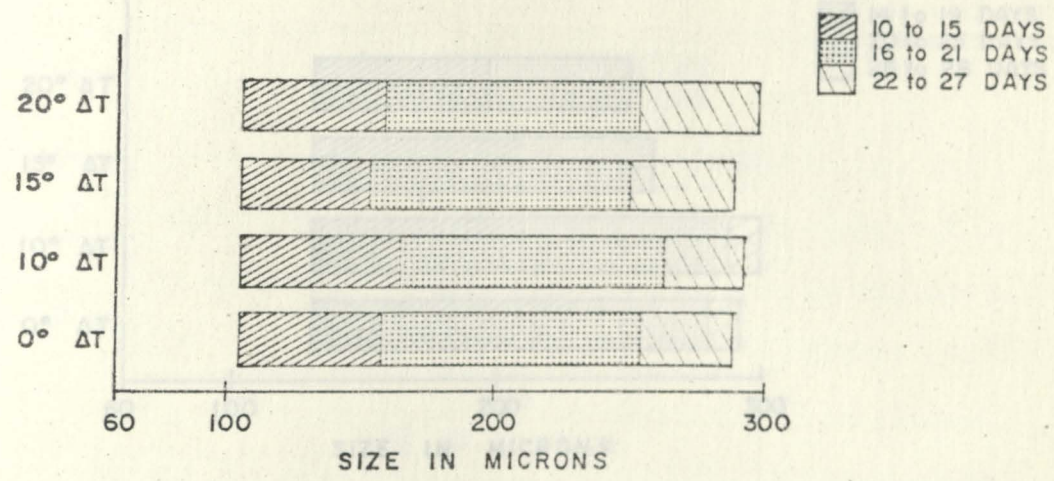


Figure 7. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of ten days.

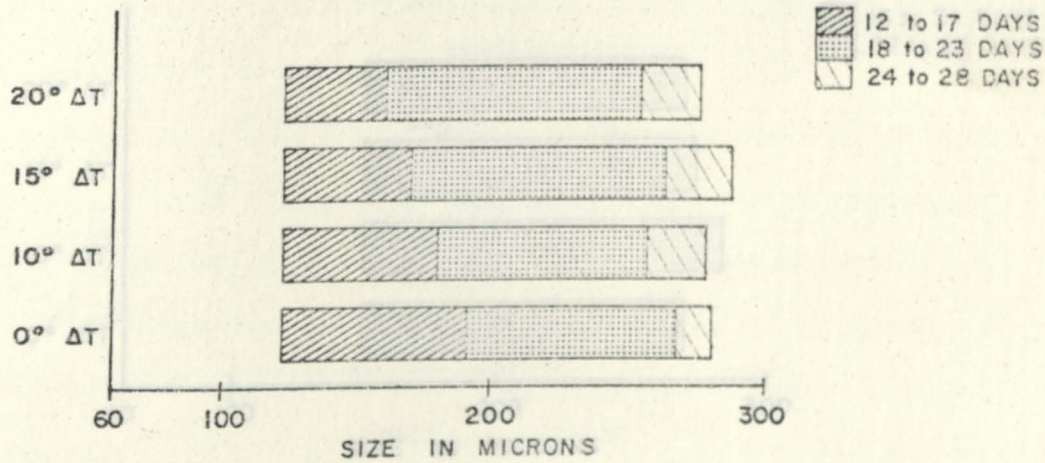


Figure 8. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of 12 days.

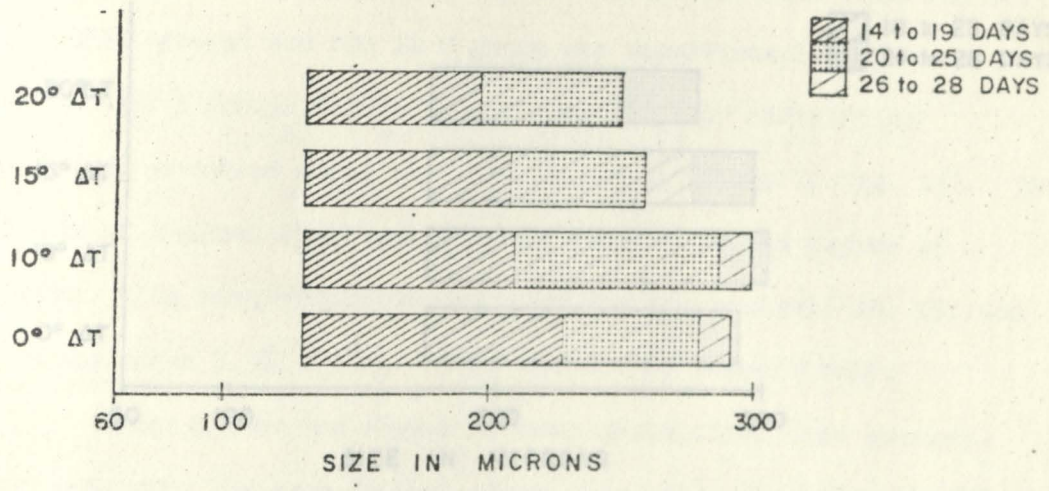


Figure 9. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of 14 days.

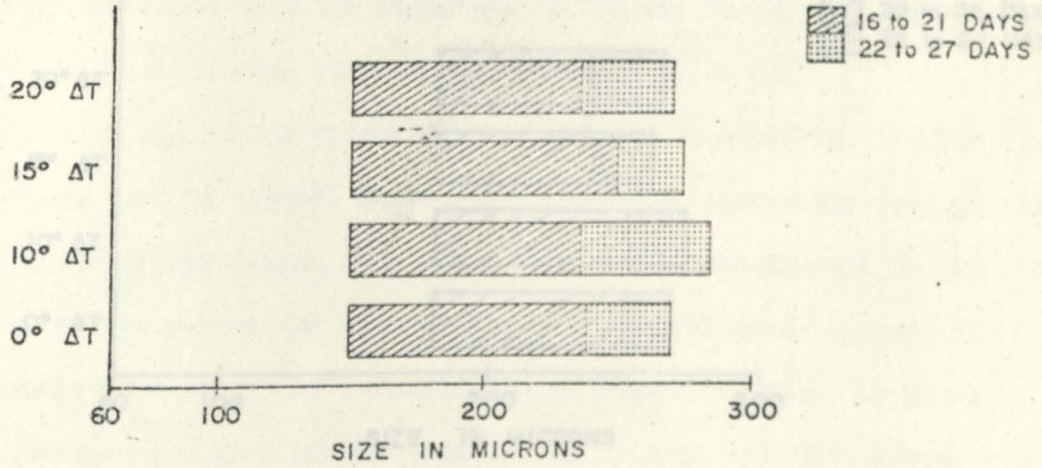


Figure 10. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of 16 days.

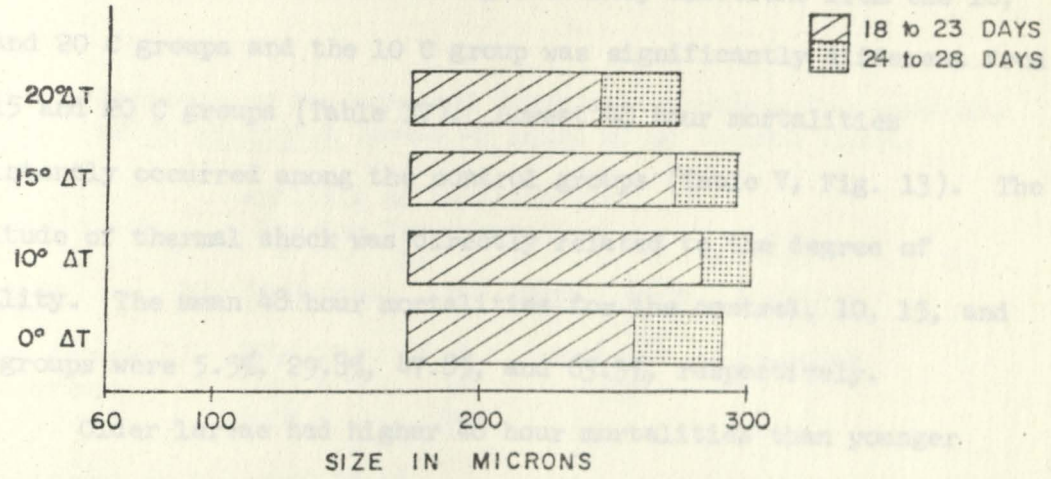


Figure 11. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of 18 days.

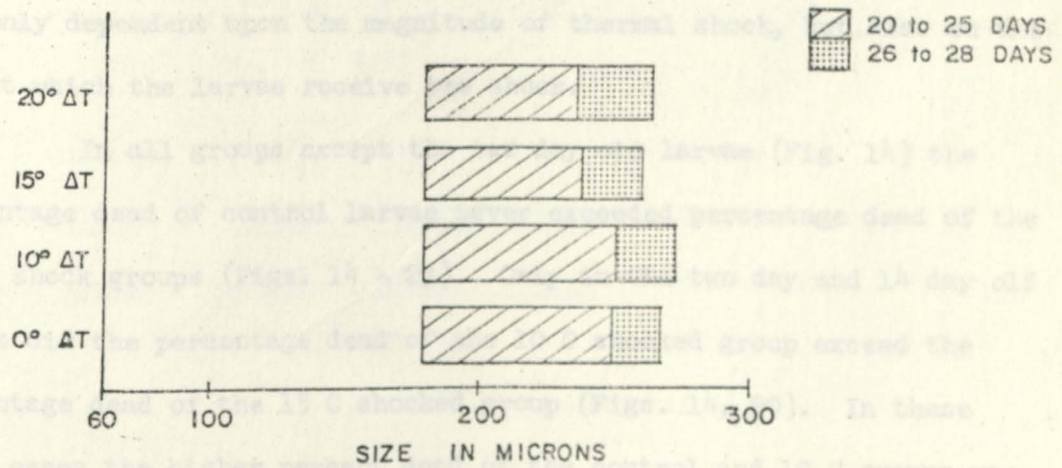


Figure 12. Five day growth intervals of C. virginica larvae subjected to thermal shock at the age of 20 days.

was significantly different from that of the other groups. At the .01 confidence level the control was significantly different from the 10, 15, and 20 C groups and the 10 C group was significantly different from the 15 and 20 C groups (Table IV). Lowest 48 hour mortalities consistently occurred among the control groups (Table V, Fig. 13). The magnitude of thermal shock was directly related to the degree of mortality. The mean 48 hour mortalities for the control, 10, 15, and 20 C groups were 5.5%, 29.8%, 47.8%, and 63.3%, respectively.

Older larvae had higher 48 hour mortalities than younger larvae receiving the same thermal shock (Fig. 13). Grouping the 48 hour mortalities of the two to six day old larvae and comparing them to the 16 to 20 day old larvae (Table V), the difference between the younger and older larval mortalities was 3.0% for controls, 31.3% for 10 C, 25.6% for 15 C, and 20.2% for 20 C shocked groups. Mortality was not only dependent upon the magnitude of thermal shock, but also on the age at which the larvae receive the shock.

In all groups except the two day old larvae (Fig. 14) the percentage dead of control larvae never exceeded percentage dead of the three shock groups (Figs. 14 - 23). Only in the two day and 14 day old groups did the percentage dead of the 10 C shocked group exceed the percentage dead of the 15 C shocked group (Figs. 14, 20). In these three cases the higher percent dead of the control and 10 C groups was due to allowable variations in sampling techniques and not attributable to thermal shock. All three cases exceeded the higher thermal shock groups by very small percentages.

Table IV. Analysis of Variance and Tukey's-w procedure for mortality of C. virginica larvae 48 hours after thermal shock.

Analysis of Variance with Arcsine Transformation

Source	Sum of Squares	Df	Mean Square	F ratio
Between groups	8835.2	3	2945.1	65.302**
Within groups	1623.6	36	45.1	
Total	10458.8	39		

**Significant at the .005 level.

Tukey's-w Procedure .01 Level

$$w_{01} = 4.74(S_{\bar{x}})$$

$$S_{\bar{x}} = \frac{s^2}{r} = \frac{45.1}{10} = 2.12$$

$$w_{01} = 9.55$$

	Control	10C	15C	20C	Groups
Means	13.32	32.82	44.01	53.18	Arcsine values
Means	5.29	29.34	48.26	64.12	Percentages

Table V. Mortality of C. virginica larvae 48 hours after thermal shock.

Age of larvae when subjected to thermal shock (days)	Control	10C	15C	20C
2	2.9	16.3	28.2	43.6
4	2.3	32.0	46.1	48.7
6	4.3	22.6	41.0	52.4
8	3.5	31.5	51.8	71.1
10	6.6	26.3	41.1	60.5
12	5.2	16.1	49.2	70.4
14	6.1	28.1	68.1	81.6
16	8.5	38.9	52.7	69.8
18	8.4	37.9	57.3	47.9
20	7.4	48.8	52.1	87.4
Mean	5.5	29.8	47.8	63.3

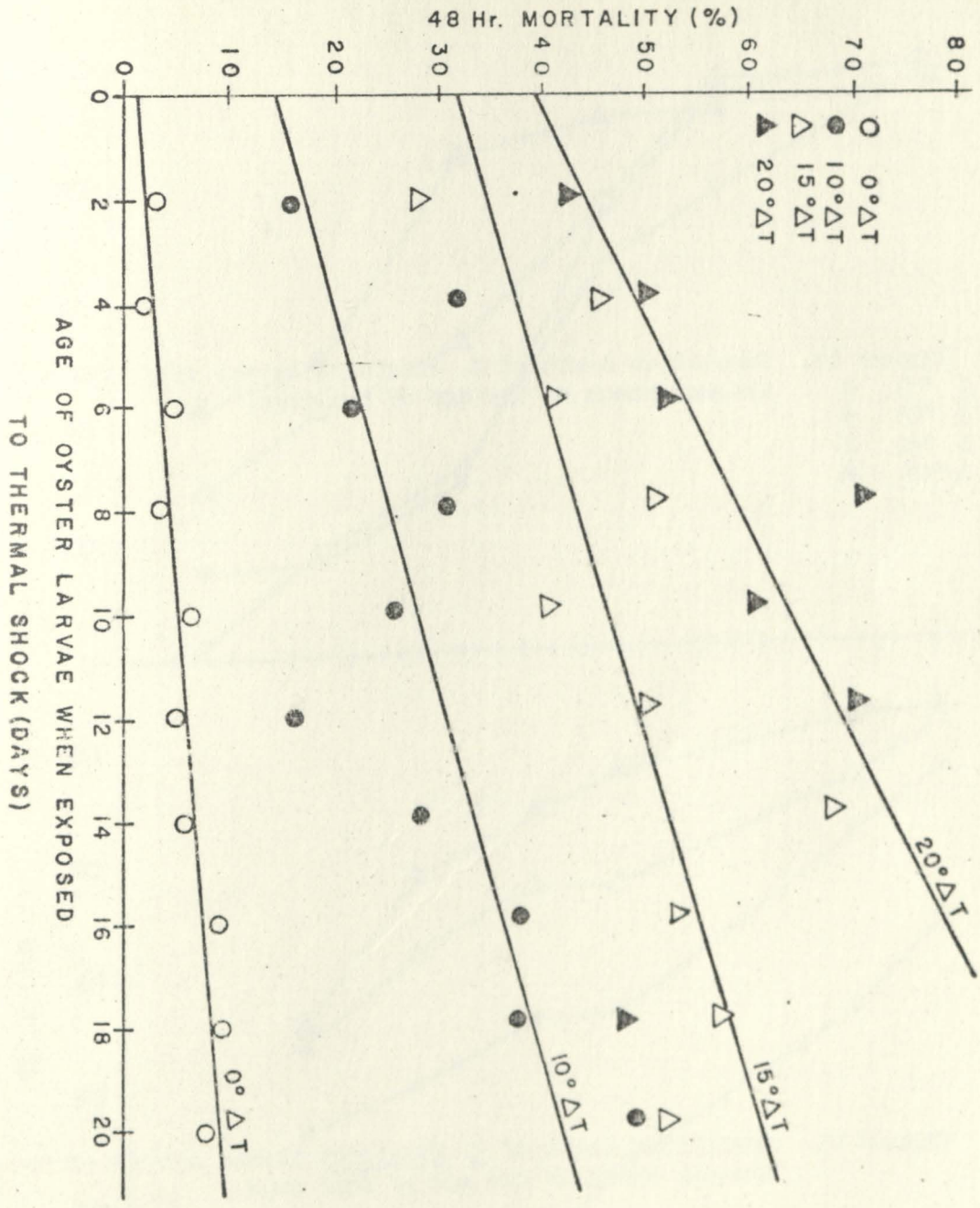
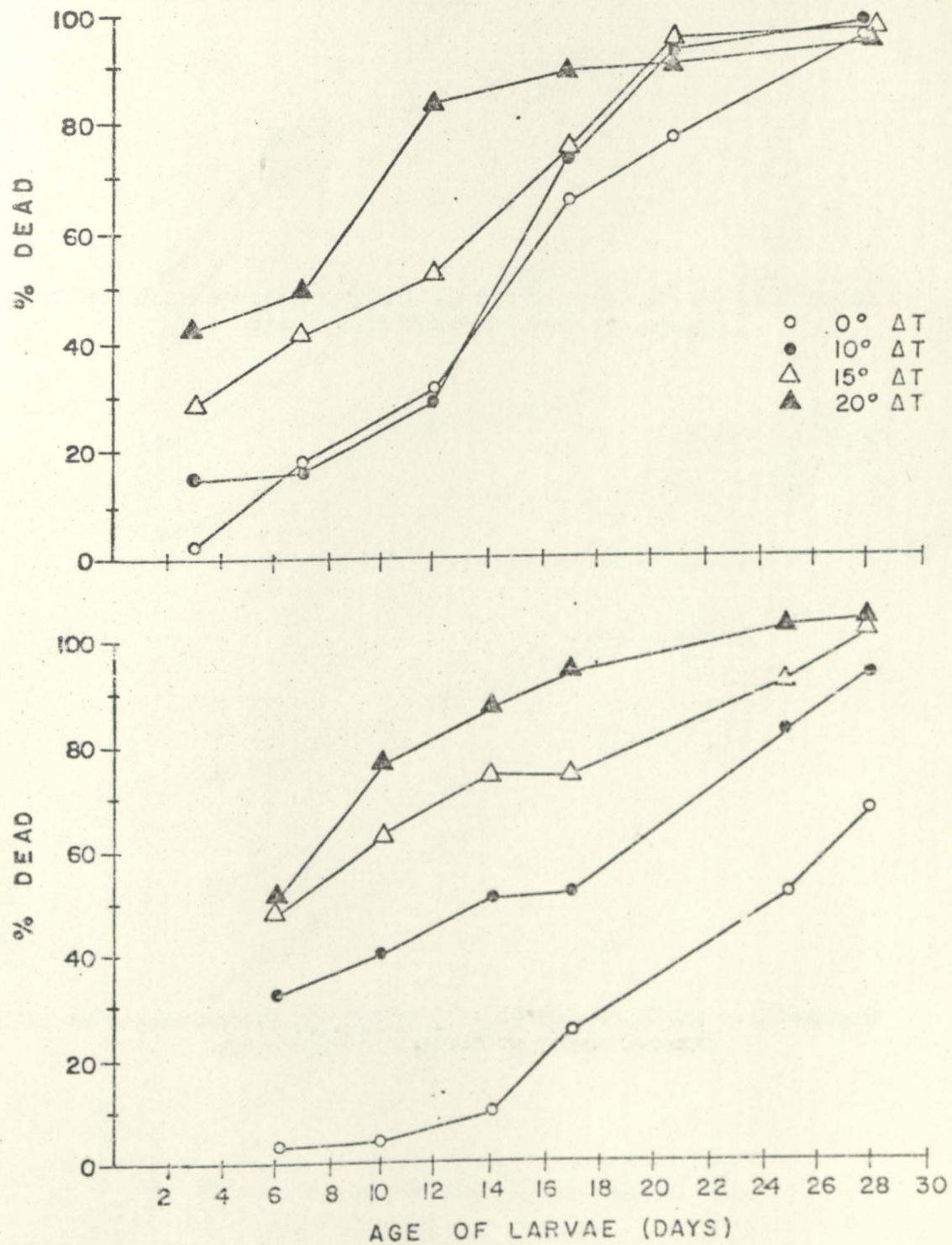


Figure 13. 48 hour mortality of C. virginica larvae subjected to thermal shocks of 10C, 15C, and 20C.

Figure 14. Cumulative death of C. virginica larvae subjected to thermal shock at the age of two days.

Figure 15. Cumulative death of C. virginica larvae subjected to thermal shock at the age of four days.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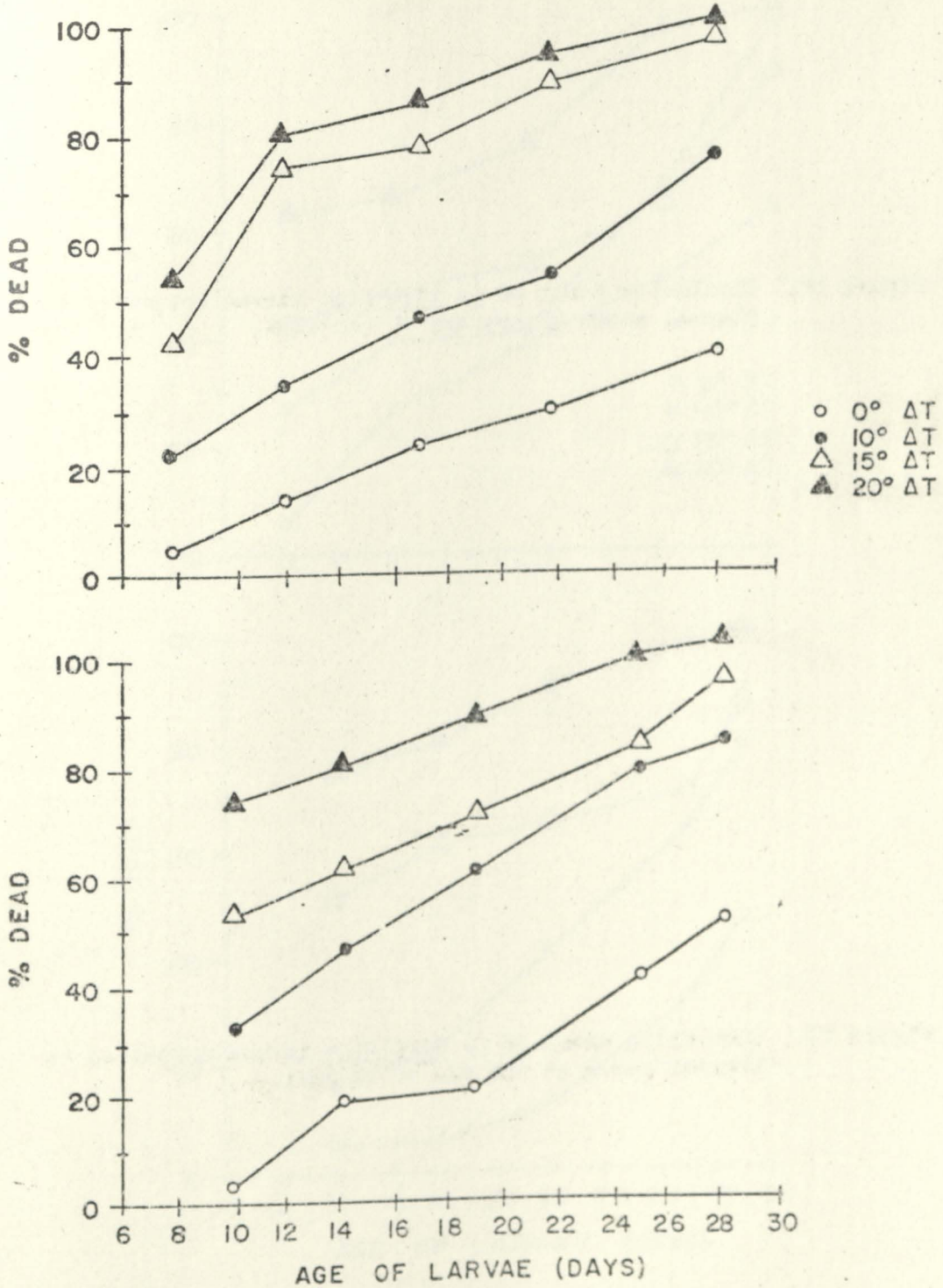
AGE OF LARVAE (DAYS)

Figure 16. Cumulative death of C. virginica larvae subjected to thermal shock at the age of six days.

Figure 17. Cumulative death of C. virginica larvae subjected to thermal shock at the age of eight days.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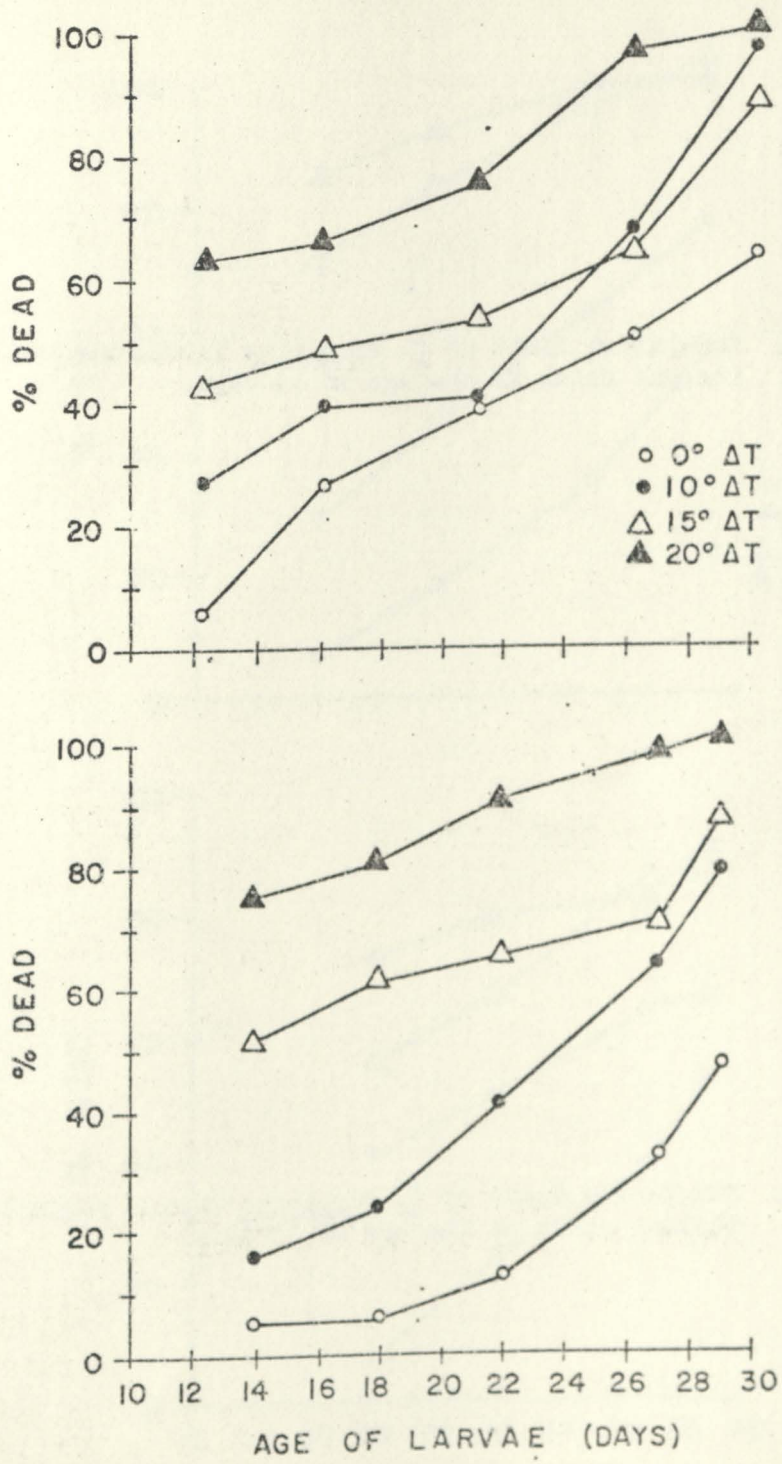
AGE OF LARVAE (DAYS)

Figure 18. Cumulative death of C. virginica larvae subjected to thermal shock at the age of ten days.

Figure 19. Cumulative death of C. virginica larvae subjected to thermal shock at the age of 12 days.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0
% DEAD
10 12 14 16 18 20 22 24 26 28 30
AGE OF LARVAE (DAYS)

Figure 20. Cumulative death of C. virginica larvae subjected to thermal shock at the age of 14 days.

Figure 21. Cumulative death of C. virginica larvae subjected to thermal shock at the age of 16 days.

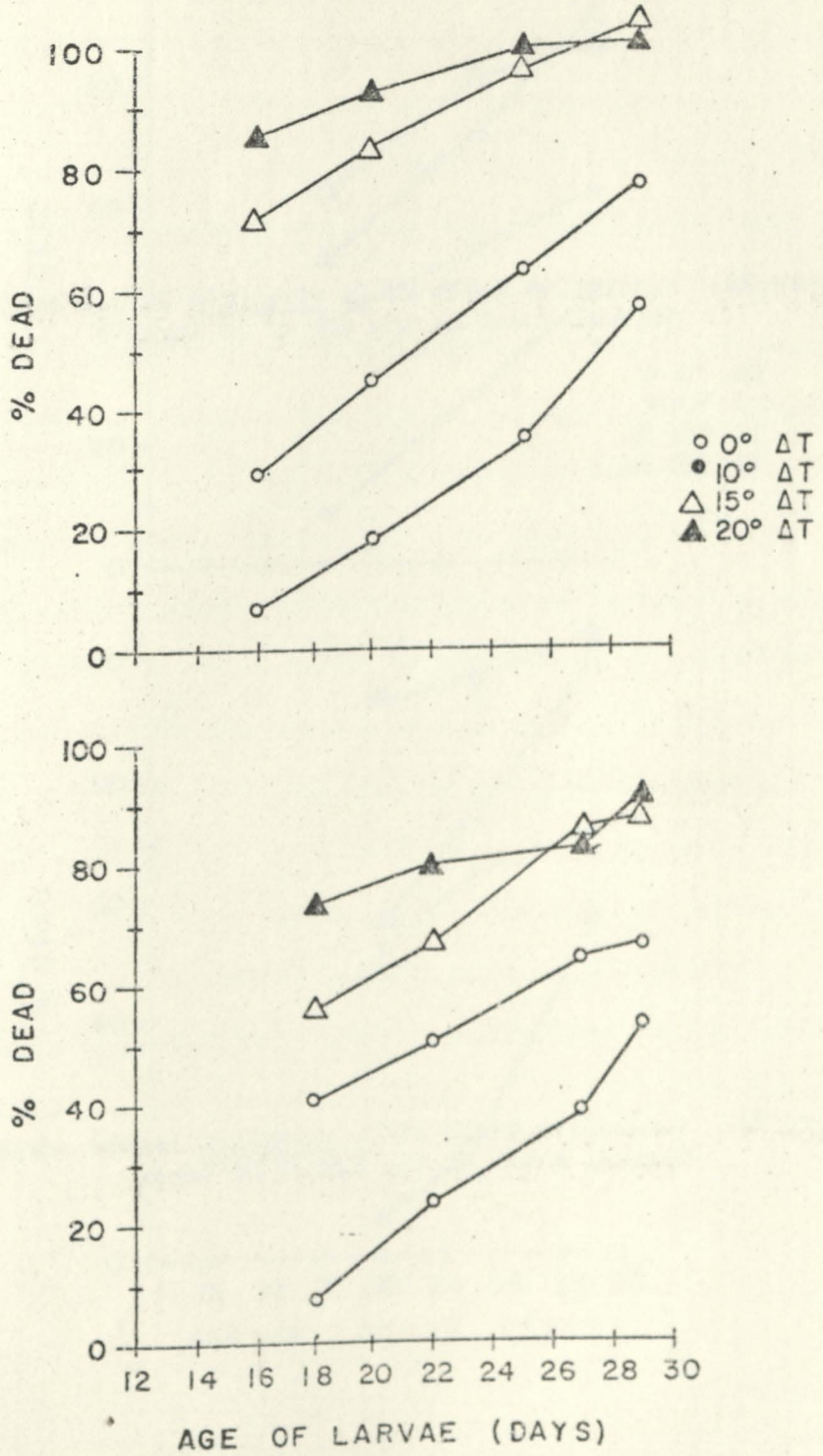
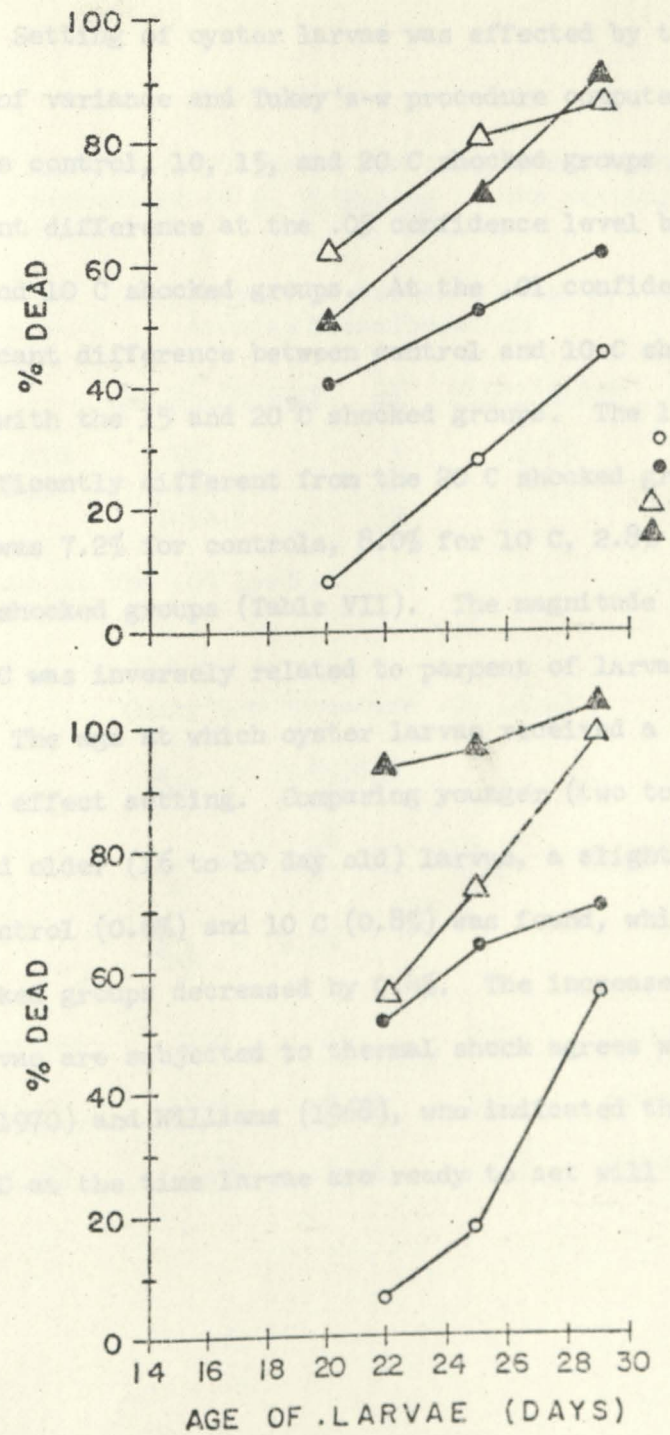

100
80
60
40
20
0
% DEAD
0° ΔT
10° ΔT
15° ΔT
20° ΔT
100
80
60
40
20
0
% DEAD
12 14 16 18 20 22 24 26 28 30
AGE OF LARVAE (DAYS)

Figure 22. Cumulative death of C. virginica larvae subjected to thermal shock at the age of 18 days.

Figure 23. Cumulative death of C. virginica larvae subjected to thermal shock at the age of 20 days.


100
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% DEAD
0° ΔT
10° ΔT
15° ΔT
20° ΔT
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60
40
20
0
% DEAD
14 16 18 20 22 24 26 28 30
AGE OF LARVAE (DAYS)

Effects on Setting

Setting of oyster larvae was effected by thermal shock. Analysis of variance and Tukey's-w procedure computed for percentage set of the control, 10, 15, and 20 C shocked groups indicated no significant difference at the .05 confidence level between setting of control and 10 C shocked groups. At the .01 confidence level there was a significant difference between control and 10 C shocked groups compared with the 15 and 20 C shocked groups. The 15 C shocked group was significantly different from the 20 C shocked group (Table VI). Mean set was 7.2% for controls, 8.0% for 10 C, 2.8% for 15 C, and 0.5% for 20 C shocked groups (Table VII). The magnitude of thermal shock above 10 C was inversely related to percent of larvae that set.

The age at which oyster larvae received a thermal shock tended to effect setting. Comparing younger (two to six day old) larvae and older (16 to 20 day old) larvae, a slight increase in the set of the control (0.6%) and 10 C (0.8%) was found, while both the 15 and 20 C shocked groups decreased by 0.4%. The increased set with age at which larvae are subjected to thermal shock agrees with Lutz, Hidu, and Drobeck (1970) and Williams (1968), who indicated that a thermal shock below 10 C at the time larvae are ready to set will increase setting.

Table VI. Analysis of Variance and Tukey's-w procedure for the percentage of C. virginica larvae setting after thermal shock.

Analysis of Variance with Arcsine Transformation

Source	Sum of Squares	Df	Mean Square	F ratio
Between groups	971.9	3	323.9	17.084*
Within groups	682.7	36	18.9	
Total	1654.8	39		

*Significant at the .05 level.

Tukey's-w Procedure .05 Level

$$w_{05} = 3.80(S_{\bar{x}})$$

$$S_{\bar{x}} = \frac{s^2}{r} = \frac{18.9}{10} = 1.38$$

$$w_{05} = 5.24$$

	Control	10C	15C	20C	Groups
Means	15.07	15.57	8.90	3.55	Arcsine values
Means	6.79	7.32	2.39	0.39	Percentages

Table VII. Percentage of C. virginica larvae setting after thermal shock.

Age of larvae when subjected to thermal shock (days)	Control	10C	15C	20C
2	3.4	1.1	0.6	0.0
4	6.8	6.6	4.7	1.4
6	4.3	6.6	1.6	0.6
8	4.0	5.5	1.8	0.0
10	16.7	23.6	9.5	2.3
12	8.6	8.6	3.4	0.1
14	11.8	10.8	1.1	0.1
16	7.2	6.2	2.0	0.3
18	4.9	5.0	3.2	0.2
20	4.2	5.6	0.5	0.2
Mean	7.2	8.0	2.8	0.5

CONCLUSIONS

1. The mean growth rate of Crassostrea virginica larvae was not significantly affected by a thermal shock of 10 or 15 C. Such shocks temporarily retarded the growth rate, but surviving larvae recovered in six to seven days. A thermal shock of 20 C permanently impaired growth rates of the oyster larvae.

2. Mortality was significantly increased by thermal shocks of 10, 15, or 20 C. Mortality was significantly higher after a 20 C shock than after a 15 C shock. Mortality after a 15 C shock was significantly higher than after a 10 C shock. Older larvae exhibited a higher mortality than younger larvae receiving the same thermal shock.

3. Setting of oyster larvae was unaffected by a thermal shock of 10 C. Shocks of 15 and 20 C significantly decreased setting, with the 20 C shock causing a significantly lower set than the 15 C shock.

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VITA

Robert James Diaz

Born in Chester, Pennsylvania, 16 October 1946. Graduated from St. James High School, Chester, Pennsylvania, June 1964; B.A. degree in biology from LaSalle College, Philadelphia, June 1968. Candidate for Master of Science at the Virginia Institute of Marine Science and the University of Virginia, 1968-1971.