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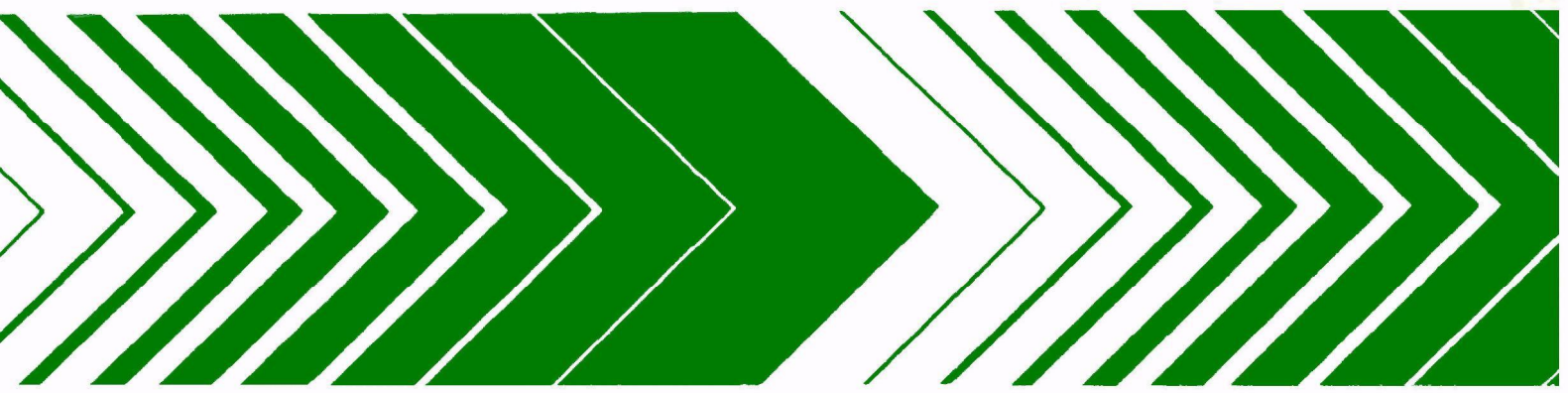
# Effects of Thermal Pollution on Pelagic Larvae of Crustacea



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# EFFECTS OF THERMAL POLLUTION ON PELAGIC LARVAE OF CRUSTACEA

by

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Grant R-800981

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## FOREWORD

The Environmental Research Laboratory of the U.S. Environmental Protection Agency is located on the shore of Narragansett Bay, Rhode Island. In order to assure the protection of marine resources, the laboratory is charged with providing a scientifically sound basis for Agency decisions on the environmental safety of various uses of marine systems. To a general extent, this requires research on the tolerance of marine organisms and their life stages as well as the tolerance of ecosystems to many forms of pollution stress.

This report describes a three-year study undertaken to determine the environmental requirements for development of pelagic life stages of some epibenthic crustaceans from the coastal and primarily estuarine environments. The effects of temperature changes on the development, metabolism and survival of the larval stages of the crustaceans are presented.

Tudor T. Davies
Director

ABSTRACT

Studies have been conducted to determine the effects of temperature singly and in combination with other environmental factors on the developmental and survival rates, metabolic adaptation and tolerance limits of larvae of selected epibenthic crustaceans from the New England region. Six species, Cancer irroratus, Cancer borealis and Homarus americanus from the coastal area (high salinity), and Palaemonetes pugio, Pagurus longicarpus and Rhithropanopeus harrisii, from the primarily estuarine region (variable salinity) were used for this study. Larvae of each species were cultured at various combinations of temperature and salinity to establish the combination contributing to highest survival rates and to determine the limits for complete development of each species. Temperature and salinity limits and the optimal combination for development varied interspecifically. Generally, coastal species had a more restrictive temperature range for complete development than estuarine species.

The effects of daily cyclic temperatures vs. a comparable constant regime on the development and survival rates of larvae were variable. Survival of larvae of C. irroratus cultured under certain daily cyclic regimes was better. In contrast, larvae of P. pugio cultured under daily cyclic regimes showed no significant differences in either survival or developmental rate when compared to those at constant temperatures.

Metabolic responses of C. irroratus, H. americanus and P. pugio larvae cultured at temperature and salinity combinations optimal for their maximum survival rate were determined over a graded series of test temperatures. Larval stages of the three species exhibited thermal sensitivity ($Q_{10}>2$), insensitivity or compensation ($1<Q_{10}<2$) and depression ($Q_{10}<1$) of metabolic rates over the range of tested temperatures. The patterns of metabolic response to temperature varied inter- and intraspecifically. Generally, the larvae of coastal species C. irroratus and H. americanus were metabolically active over a narrower temperature range compared to the primarily estuarine species, P. pugio. Metabolic-temperature response patterns of the larval stages of each species cultured at daily cyclic temperatures were altered relative to those cultured at comparable constant temperatures. The differential effects of daily cyclic and constant temperatures were also exhibited in the fatty acid methyl esters in larval stages of H. americanus and in the enzyme systems examined in the larval stages of C. irroratus.

Acute temperature and low dissolved oxygen tolerances were determined for larval stages of C. irroratus, H. americanus and P. pugio. The thermal tolerance limits for the larval stages of the primarily estuarine grass shrimp, P. pugio were higher compared to those for larvae of coastal species, C. irroratus and H. americanus. When temperature and low dissolved oxygen

stresses were combined, the thermal tolerance limits of C. irroratus larval stages were altered.

Environmental thermal alterations can affect survival, developmental rates and metabolism of developing larvae of crustaceans. The specific effects of thermal alteration will vary during the course of larval development in a manner characteristic to each species. The nature of these effects will be influenced by the organisms recent thermal history, as illustrated by the higher survival rate of larvae reared under cyclic temperature conditions than at a single constant temperature. Further, these effects are modified as other environmental parameters, such as dissolved oxygen and salinity interact with temperature. Generally, estuarine species have adaptive capacities for development and growth over a wider range of environmental conditions than coastal species.

This report was submitted in fulfillment of Grant number R 800981 under the sponsorship of Environmental Protection Agency. Work was completed as of September 1976.

CONTENTS

FIGURES

FIGURES (cont'd)

TABLES

TABLES (cont'd)

## ACKNOWLEDGEMENTS

The financial support of the Environmental Protection Agency under Grant No. R-800981 is gratefully acknowledged. Dr. Don Miller, Project Officer, has been particularly helpful in carrying out this project.

The project was carried out at the Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island under the direction of A. N. Sastry with the assistance of Dr. Sandra L. Vargo. Technical assistance was provided by Ms. Shirley Barton, Ms. Sharon Pavignano, Ms. Joyce Blaisdell, Mr. John Laczak, Mr. Ross Ellington and Mr. John McCarthy. Ms. Sharon Pavignano provided invaluable assistance in the preparation of the final report.

# SECTION 1

## INTRODUCTION

The possible effects of thermal alterations of the coastal and estuarine environments from conventional and nuclear power plants has become a concern to the public during the past several years. The constraints of thermal increase in these bodies of water are determined by consideration of the effects on the biotic communities. The adaptive capacities of organisms either to a direct increase of water temperature, or the changes in the physico-chemical properties of sea water due to increased temperature are important considerations for determining water quality criteria. An important issue then in thermal pollution of the environment is to assess its short and long term impact on biotic communities which may eventually result in alterations in the structure and functioning of these ecological systems. To develop the capability for assessment and prediction of thermal alterations of the environment, there is a need for a comprehensive understanding of the effects of thermal changes, singly or in combination with other factors, on all the life stages of populations within the communities.

An extensive body of literature evaluating the effects of thermal pollution on organisms has been already published (Clark, 1969; Jensen, et al., 1969; Krenkel and Parker, 1969; Nylor, 1969; Hargis and Warinner, 1970; Gibbons and Shiritz, 1974; Esch and McFarlane, 1976; Coutant and Talmage, 1977). It is generally recognized that temperature changes in the environment affect metabolism, growth, reproduction and development, and activity and behavior of the organisms. The functional activities of organisms are performed within a temperature range characteristic to each species (Prosser, 1971; Vernberg and Vernberg, 1972). Within the tolerance range, physiological rate processes are altered relative to changes in the ambient water temperature. However, many organisms have capabilities to regulate their phsiological rate functions within a certain temperature range (Bullock, 1955; Hazel and Prosser, 1974; Wieser, 1973). These adaptations to changes in the thermal environment play an important role in their ecology and distribution. At either extreme of the tolerance range, there is a point beyond which the organisms are unable to survive. The adaptations which favor regulation of physiological rate processes within the tolerance range and the limits may vary with the stage of life cycle and also with interactions of other environmental factors (Sastry and Vargo, 1977). The synergistic interaction of multiple factors in the environment makes it difficult to describe and evaluate the complex relationship between organisms and their environmental alterations under natural conditions. One approach to determine the effects of temperature changes on organisms is to measure alterations in their physiological rate processes within the tolerance range as well as measuring the thermal limits for survival. Since a number of physical, chemical and biological factors interact with the organisms under natural conditions to

affect their distribution, abundance and survival, laboratory studies of temperature effects must be extrapolated with caution to a field situation, keeping in view the experimental limitations. None the less, laboratory studies provide an understanding of the effects of environmental alterations on the adaptive capacities of organisms and give some insight into the probable consequences to organisms under natural conditions.

Effects of thermal alterations in the environment on adaptations of adult marine organisms have been extensively studied (Kinne, 1962, 1963, 1970; Vernberg and Vernberg, 1971; Prosser, 1971; Wieser, 1973). However, the adaptations for functional activities and tolerance limits of the planktonic early life stages of benthic organisms relative to changes in the thermal environment are not well known. Pelagic larvae are a vulnerable life cycle link and their survival through complete development is important for successful recruitment to the adult populations (Thorson, 1950; Meleikovsky, 1970). During pelagic existence, the larvae are exposed to varying combinations of temperature, salinity, light, food and other factors. Of these factors, temperature, acting either singly or in combination with others, often is of major importance to the development and growth, survival and distribution (Costlow and Bookhout, 1964; Sastry and Vargo, 1977). In the present project, studies have been conducted to determine the effects of temperature, singly and in combination with other factors, on development and survival, tolerance limits and metabolic adaptation of larvae of several epibenthic crustaceans from the coastal (high salinity) and predominantly estuarine waters of the New England region.

## SECTION 2

## CONCLUSIONS

Thermal alterations affect the development and growth, metabolism and survival of crustacean larvae. The limits as well as the optimal conditions for larval development are modified by the thermal history of eggs prior to hatching, the stage of larval development and geographic origin of the population. Different combinations of temperature and salinity interact to affect larval development and survival. A species may exhibit an optimal combination for survival and as environmental conditions deviate from this optimum, survival is reduced. Limits, as well as optimal combinations for development, vary interspecifically. Generally, complete development for coastal (high salinity) species, Cancer irroratus, Cancer borealis and Homarus americanus was limited to a narrower range of temperature and salinity than predominantly estuarine species, Palaemonetes pugio, Pagurus longicarpus and Rhithropanopeus harrisii.

Time for complete development and percent survival can also be influenced by use of a daily cyclic temperature regime. Larvae of C. irroratus cultured under suitable daily cyclic regimes showed increased survival compared to those at comparable constant temperatures. The effects of daily cyclic temperatures can vary interspecifically, however. For P. pugio, development and survival rates were not significantly different under daily cyclic and comparable constant temperatures.

Within the thermal tolerance range of a species, the metabolic responses to temperature may vary, but each species will have a characteristic overall response. Larval stages of each species exhibited capacities for metabolic rate compensation ($1<Q_{10}<2$) over a portion of the temperature range. Generally, the larvae of coastal species are metabolically active over a relatively narrower temperature range than the estuarine species. The metabolic-temperature response patterns of the larvae are also influenced by the culture conditions: cyclic or a comparable constant temperature. The alterations in metabolic adaptation of larvae cultured under daily cyclic temperatures were underlined by qualitative and quantitative changes in fatty acid methyl esters in H. americanus larvae and changes in specific activities of the enzyme systems examined in C. irroratus larvae.

Acute temperature tolerance limits of laboratory cultured larvae varied intra- and interspecifically, with estuarine species tolerating higher temperatures than coastal species. These thermal tolerance limits remained fairly constant for all C. irroratus larval stages, while some of H. americanus and P. Pugio stages showed differential sensitivity when tested under saturated dissolved oxygen conditions.

## SECTION 3

## RECOMMENDATIONS

Culture studies of larvae of several predominantly estuarine and coastal (high salinity) epibenthic crustaceans show that complete development occurs within a temperature and salinity range which is characteristic of each species. Limits, as well as the optimal combination for survival, vary interspecifically. Limits, as well as optimal conditions for maximum survival also varied with the prior environmental history of eggs (i.e. for temperature and salinity), stage of larval development and geographic origin of the populations. Developmental and survival rates are also altered under daily cyclic culture temperatures relative to those at constant temperatures. The effects of fluctuating temperatures on development and survival were more evident for coastal species than estuarine species.

Larvae of the crustaceans examined in this study showed abilities for metabolic regulation over a temperature range characteristic to each species. The metabolic rate of larvae showed sensitivity, insensitivity or compensation and depression of metabolic rate over the gradient of temperatures tested. Generally, the larvae of estuarine species were metabolically active over a broader temperature range than coastal species. The metabolic-temperature response patterns of larvae also varied with the stage of development of each species. These patterns were altered for larvae cultured under daily cyclic temperatures relative to those at constant temperatures. The alterations in metabolic responses of larvae cultured under daily cyclic regimes were underlined by qualitative and quantitative changes in fatty acid methyl esters and also in the specific activities of enzyme systems examined.

The thermal tolerance limits of larvae were found to be generally higher for the estuarine species than coastal species. The tolerances of larvae are modified when temperature and dissolved oxygen stress were combined.

Biochemical and physiological responses of developing larvae to temperature and other interacting factors need to be examined when evaluating the effects of thermal alterations on the development and growth. In addition, the effects of varying environmental temperatures and their interaction with other physioco-chemical factors on metabolism, development and growth of larvae can also provide some valuable information on their abilities for adaptation to altered thermal environment. Studies on larvae of species occuring in different habitats and as well those breeding in different seasons would provide valuable information regarding the probable consequences of environmental thermal alterations on the recruitment of young to the adult populations in a geographic region.

## SECTION 4

## MATERIALS AND METHODS

### CULTURE OF LARVAE

Larvae of six species of crustaceans, Cancer irroratus, Cancer borealis, and Homarus americanus from the coastal zone (high salinity), and Pagurus longicarpus, Palaemonetes pugio and Rhithropanopeus harrisii from predominantly estuarine waters in Narragansett Bay have been cultured in the laboratory. Details of their habitat, temperature and salinity conditions are summarized in Table 1. The general methods for incubation and larval cuture are the same as described in detail by Sastry (1970) and Sastry and Vargo (1977). Ovigerous adults were brought to the laboratory, eggs removed and incubated until hatching in 30 o/oo salinity at a temperature suitable for each species (Table 2). In the cases of H. americanus and P. pugio, eggs were not removed and the ovigerous animals were held at the incubation temperatures until hatching.

Within 24 hours from hatching of eggs in the laboratory, the larvae were transferred to different temperature and salinity combinations for rearing. Larvae were reared individually in compartmented plastic boxes containing filtered sea water or in the case of H. americanus in glass crystallizing dishes containing sea water. Within 24 hours after hatching of eggs, the larvae were maintained in Sherer controlled environmental chambers and were exposed to a 14:10 LD cycle. The sea water was changed on alternate days and the larvae were fed daily on a diet of freshly hatched Artemia salina nauplii. Molting and deaths were recorded daily to quantify the patterns of survival to complete development for each species at the various conditions. Temperature and salinity effects were determined using a factorial design to produce graded series of responses to constant 10, 15, 20 and 25 C and salinities 10, 15, 20, 25, 30 and 35 o/oo. The factorial design allowed estimation of the range of overall effects and the interaction between different experimental factors (Alderdice, 1972).

The morphological descriptions of the larval stages of each species except C. irroratus and C. borealis have been reported in the literature. Drawings and descriptions of larval stages of C. irroratus and C. borealis were made from larvae of known stages cultured in the laboratory under optimal temperature and salinity conditions and placed in Permount on a slide. Whole mounts were made of fresh material, and appendages also dissected from each stage and similarly mounted. Scale drawings were made of whole mounts and larval appendages with the aid of camera lucida (Sastry, 1977a, b). The descriptions of these larvae and those of other species available from literture were used for recognizing different stages in the development of a species in conducting the experimental work on their physiological and bio-

Table 1 Distribution, ecology and breeding period of seven species of crustaceans used in the study

| Species | Geographical Distribution | Habitat (Williams,1965) | Narragansett Bay and Vicinity: Collection Locality | Breeding Period | Habitat Temperature °C and Salinity (o/oo) during breeding period |
|---|---|---|---|---|---|
| Pagurus longicarpus | Nova Scotia to Northern Florida and from Sanibel Island, Florida to Texas | Common on harbor beaches, and in shallow littoral on a variety of bottoms. | Boat basin | Late April to mid-June | 7.5-17.0 C<br>31 o/oo |
| Palaemonetes pugio | Massachusetts to Texas | Estuarine waters especially in submerged vegetation | Bissell's Cove | Mid-June to early September | 20-25 C<br>10-30 o/oo |
| Rhithropanopeus harrisii | Canada to Mexico, northeast Brazil, introduced to West Coast of U.S. and in parts of Europe | Estuarine, found in places always providing shelter | Narrow River | Mid-June to late August | 22-27 C<br>13-26 o/oo |
| Cancer irroratus | Labrador to South Carolina | Low water mark to 600 m, shallow bay in north and deep waters in south | Narragansett Bay | April to early July | 6-19 C<br>31 o/oo |
| Cancer borealis | Nova Scotia to Tortugas, Florida and Bermuda | Between tides in rocks to 870 m; shallow bays in north and deep waters in south | Narragansett Bay | July | 18-23 C<br>31 o/oo |
| Homarus americanus | Nova Scotia to New Jersey | Sub-littoral rocky bottom | Narragansett Bay and vicinity | July | 18-23 C<br>31 o/oo |
| Panopeus herbstii | Massachusetts to Brazil; Bermuda | Estuarine, bottom composed of soft mud and oyster shells | Pawcatuck River | July | 18-23 C<br>31 o/oo |

chemical responses to temperature changes.

For experimental work on the physiology and biochemistry, larvae of each species were mass cultured in 12 cm diameter finger bowls under previously determined optimal temperature and salinity combination producing the maximum survival. The larvae were transferred frequently to fresh sea water and provided with a diet of freshly hatched Artemia salina nauplii as previously described.

TABLE 2 EGG INCUBATION AND LARVAL CULTURE CONDITIONS USED FOR SIX SPECIES OF CRUSTACEA

| Species | Egg Incubation | Larval Culture Conditions | | |
|---|---|---|---|---|
| | | Temperature (°C) | Salinity (o/oo) | Number of Combinations |
| Cancer irroratus | 15°C, 30 o/oo | 10,15,20,25 | 10,15,20,25,30,35 | 24 |
| Cancer borealis | 15°C, 30 o/oo | 10,15,20,25 | 10,15,20,25,35 | 24 |
| Homarus americanus | 20°C, 30 o/oo | 10,15,20,25 | 15,20,25,30,35 | 20 |
| Pagurus longicarpus | 15°C, 30 o/oo | 10,15,20,25 | 15,20,25,30,35 | 20 |
| Rhithropanopeus harrisii | 20°C, 25 o/oo | 20,25,30 | 10,15,20,25,30,35 | 18 |
| Palaemonetes pugio | 20°C, 30 o/oo | 10,15,20,25,30 | 5,10,15,20,25,30,35,40 | 40 |

## Effects of Daily Cyclic Temperatures on Larval Development

Effects of daily cyclic and comparable constant temperatures on developmental and survival rates of C. irroratus larvae hatched in 30 o/oo at 15 C and P. pugio larvae hatched in 30 o/oo salinity at 20 C were determined. The larvae were reared in 10-20, 15-25, 12.5-17.5 and 17.5-22.5 C daily cyclic temperatures and comparable constant 15 and 20 C under a 14:10 LD photoperiod. The same culturing methods were used as for the temperature and salinity studies cited above. Molting and deaths were recorded daily to determine the patterns of survival and duration of development.

## Metabolic Responses of Larvae to Temperature

The temperature effects on metabolism were determined by measuring the oxygen consumption rates for the larval stages of C. irroratus, H. americanus and P. pugio over a graded series of temperatures. For the metabolic rate determinations, larvae from mass cutures of C. irroratus in 30 o/oo salinity at 15 C and H. americanus and P. pugio in 30 o/oo salinity at 20 C were used. The oxygen consumption rates of C. irroratus and P. pugio were measured with all glass differential microrespirometers (Sastry and McCarthy, 1973). Metabolic rates were measured using 10-12 individuals of the first, second and third stage zoeae, 4-5 individuals of 4th and 5th stage zoeae and one of megalops for C. irroratus. For P. pugio, one to four individuals of each larval stage, depending upon the stage of development, were introduced into each flask for respiration measurements. The metabolic rates of H. americanus

larvae were measured for 4-5 individuals of first, second and third stages, and one or two individuals of the fourth stage larvae introduced into each flask. A number of replicate runs (3-12) were made for larval stages of each species over a graded series of temperatures between 5 and 30 C. Larvae were then weighed to the nearest microgram on a Cahn electrobalance. The mean oxygen consumption rate and the standard deviation were computed. The regression analysis of oxygen consumption as a function of weight of each life cycle stage was performed and tested for significance by F test. The mean weight-specific oxygen consumption rates, or those values determined from the regressions when those were significant, were plotted against temperature to represent the metabolic temperature response patterns. These values were used to compute the $Q_{10}$ for 5 C test temperature intervals to reveal changes in metabolic response to temperature.

Fatty Acid Methyl Esters

A known weight of each larval stage of C. irroratus and H. americanus were placed in 25 ml centrifuge tubes with teflon lined screw caps. Ten ml of 0.5 N KOH in methanol, and 5.0 ml of benzene were added to each tube, the tubes were flushed with nitrogen, sealed and heated at 100 C for 30 minutes with shaking for 10 min. The saponified samples were cooled, 5.0 ml of 0.3 N HCL were added, and the tubes were shaken and centrifuged. The benzene layer was drawn off and transferred to 50 ml pearshaped flasks and the aqueous methanol phases were twice re-extracted with 5 ml portions of petroleum ether. The petroleum-benzene extracts were evaporated to dryness under reduced pressure.

The extract residues were transferred to screw cap centrifuge tubes with teflon lined caps, using 1 ml methanol and 1 ml of benzene. One ml of $BF_3$ methanol was added to each tube, the tubes were flushed with nitrogen, sealed and heated in boiling water for 5 minutes. Methyl esters were isolated by adding 4 ml of $H_2O$ and 4 ml of petroleum ether. Solvent was evaporated under reduced pressure.

Fatty acid methyl esters were separated by thin layer chromatography using Me-palmitate (16:0) and Me-decosahexaenate (22:6) as spotting standards. The silica-gel plates were developed for 30 minutes in 95:5:1 petroleum ether, ethyl ether and NaOH. After drying the plates, they were sprayed with bromophenol blue solution and the bands corresponding to the saturated and unsaturated fatty acids were scraped. The scrapings were added to a microfilter apparatus attached to an aspirator and eluted with 30 ml of $CHCl_3$. The elutant was evaporated to dryness in a 50 ml pearshaped flask. The sample material was resuspended in a small amount of $CHCl_3$.

Fatty acid methyl esters of the sample of each larval stage were analyzed by Hewlet-Packard Model No. 5 1 A Gas Chromatograph, equipped with flame ionization detectors. Fatty acid methyl esters were quantitatively identified by comparison of their relative retention time with those of standard methyl esters. The fatty acids in samples were determined by incorporating a fatty acid internal standard into the extraction with the sample prior to saponification. The peak area (height X width at half height) of the internal standard methyl esters was then compared with the peak areas

of the sample methyl esters on the gas chromatogram. The fatty acid methyl esters were expressed as weight percent. All solvents used were distilled and blank solvents showed negligible quantities of fatty acids.

Enzyme Assays

Specific activities of lactate dehydrogenase, malate dehydrogenase and glucose 6-phosphate dehydrogenase were assayed for pooled groups of larval stages of C. irroratus cultured at 10-20 C daily cycle and 15 C constant temperature. Larvae were homogenized in 1:5 (weight/volume) of 5 mM Tris-HCl (pH 7.5) and the resulting homogenate was centrifuged at 25,000 g in a refrigerated centrifuge for 20 minutes. The resulting supernatant was used as the source of enzymes. The assays were performed using the following reaction mixtures: LDH, 0.01 mM NADH, 5 mM sodium pyruvate and 50 mM Tris-HCl (pH 7.5) in a total volume of 3.0 ml;
MDH, 0.01 mM HADH, 0.2 mM oxaloacetate and 50 mM Tris-HCl (pH 7.5) in a total volume of 3.0 ml;
G6PDH, 0.15 mM NADP, 1.66 mM glucose 6 phosphate, 5 mM $MgCl_2$ and 50 mM Tris-HCl (pH 7.5) in a total volume of 3.0 ml. Enzyme assay mixtures were equilibrated to 15 C prior to assay in a Zeiss PMQ II Spectrophotometer. Enzyme activities were expressed as μ moles per mg Lowry protein.

Acute Temperature and Dissolved Oxygen Tolerances

Acute temperature and low dissolved oxygen tolerances for the larvae of C. irroratus, H. americanus and P. pugio were determined for each stage of the three species. The larval stages were placed in flat walled 25 ml T-type glass flasks designed for tissue culture, which allow direct microscopic examination of the larvae for testing. Lethal temperature limits were determined under saturated dissolved oxygen conditions by bubbling with compressed air. The tests were conducted at 2 C intervals between 27 and 33 C, using 10 larvae at each temperature. After the flasks had been equilibrated to the test temperature, the larvae were introduced and the number surviving was determined at 30, 60, 120 and 240 minute intervals. Cessation of heart beat was the criterion of death. After testing, the larvae from each flask were returned to their culture temperature and the number of survivors was determined after 24 hours. The tolerance of larval stages to low dissolved oxygen was determined by reducing the dissolved oxygen from saturation (4.2 to 6.2ml $O_2$/l) to 0.2 ml $O_2$/l at 5 test temperatures (10, 15, 20, 25 and 30 C) were used. After the test, two 60 ml oxygen samples were drawn from each flask and analyzed by modified winkler method (Carritt and Carpenter, 1966). The time intervals and criterion of death were the same. The $LD_{50}$ values for temperature and dissolved oxygen were determined by the graphical method (Goldstein, 1964). Because the tolerance to low dissolved oxygen has been measured, the greater the $LD_{50}$ value the lower the tolerance; therefore, the $LD_{50}$ value is plotted as $1/LD_{50}$. With this transformation, the higher values in the plot represent greater tolerances (Vargo and Sastry, 1977). In the temperature and low dissolved oxygen tests, tolerances of individual larval stages were highly variable for the 30 and 60 min. time sampling intervals; hence only the data for the 120 and 240 min. time intervals are given. This variability is probably due to differing activity levels resulting from handling when the larvae were transferred to the test flasks.

## SECTION 5

## EXPERIMENTAL RESULTS

### LARVAL DEVELOPMENT

The methods for culture and descriptions of larval stages during complete development of C. irroratus and C. borealis were not previously available. Therefore, the larvae of both these species have been cultured and morphological features of the larval stages have been described (Sastry, 1970; 1977 a, b). Larval development of both species includes five zoeal stages and a megalops stage before metamorphosis to the crab stage (Figs. 1 and 2). Larval stages of H. americanus have been previously described (Herrick, 1896; Hadley, 1909). The development of this species includes four planktonic larval stages. Connolly (1925) described the four zoeal stages and a megalops stage in the development of R. harrisii. Larvae of P. pugio were described by Broad (1957). In the present study, the number of larval stages in the development of this species varied from 6 to 14 in various combinations of temperature and salinity (Table 3). Larval stages of P. longicarpus cultured in the laboratory have been described by Roberts (1970). The larvae pass through four zoeal stages and a megalops stage before metamorphosis to the post-larval stage.

TABLE 3. VARIABILITY IN THE NUMBER OF LARVAL STAGES DURING DEVELOPMENT OF PALAEMONETES PUGIO CULTURED AT DIFFERENT COMBINATIONS OF TEMPERATURE AND SALINITY

| Temperature C | Salinity 0/00 10 | 15 | 20 | 25 | 30 | 35 |
|---|---|---|---|---|---|---|
| 15 | 8-14 | 7-12 | 8-12 | 8-12 | 9-12 | 8-14 |
| | (10) | (10) | (10) | (10) | (10) | (10) |
| 20 | 6-9 | 6-9 | 6-9 | 6-10 | 7-11 | 6-11 |
| | (8) | (8) | (8) | (8) | (8) | (8) |
| 25 | 7-11 | 6-11 | 6-11 | 6-10 | 6-9 | 7-12 |
| | (8) | (9) | (8) | (8) | (7) | (10) |

Numbers in parenthesis indicate the model frequency

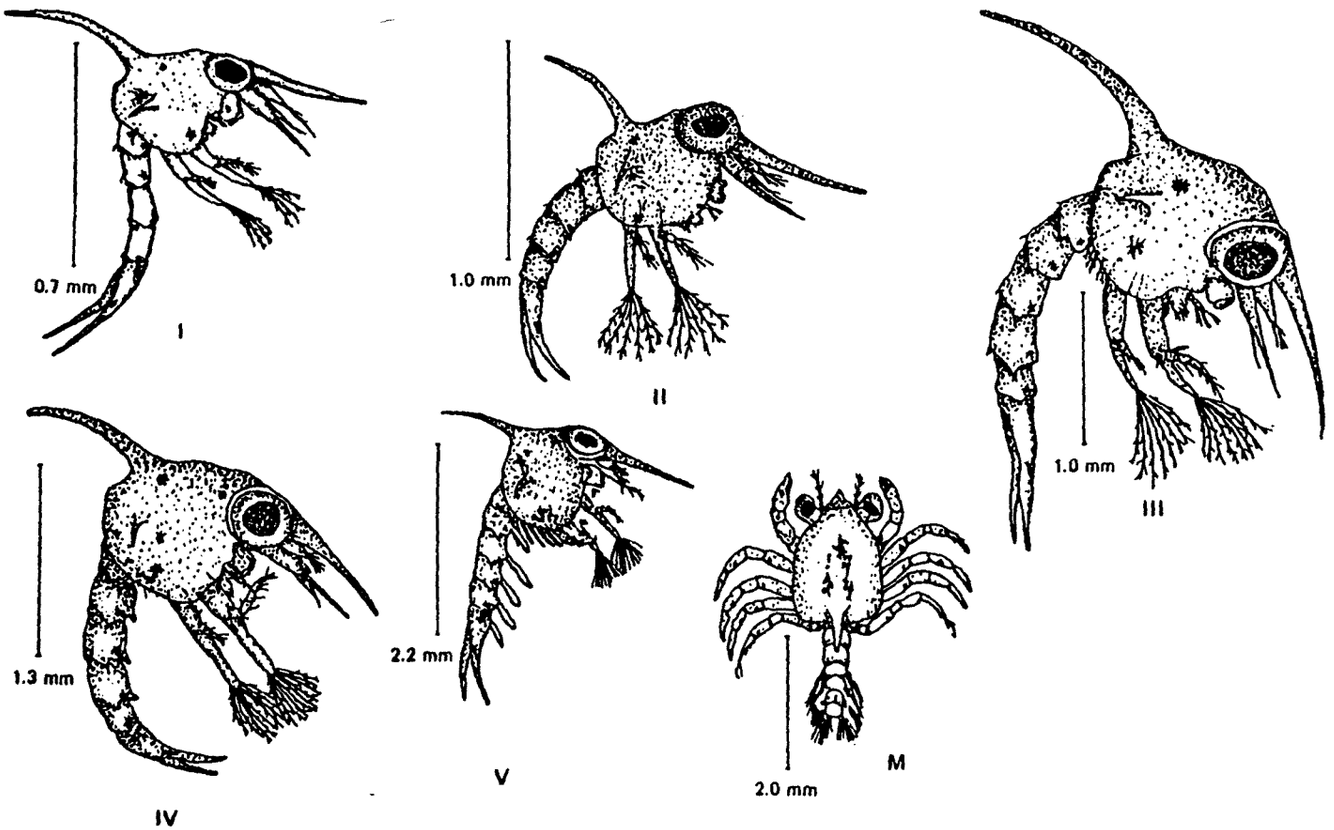


Figure 1. Life cycle stages of C. irroratus I to V, zoeal stages; M. megalops.

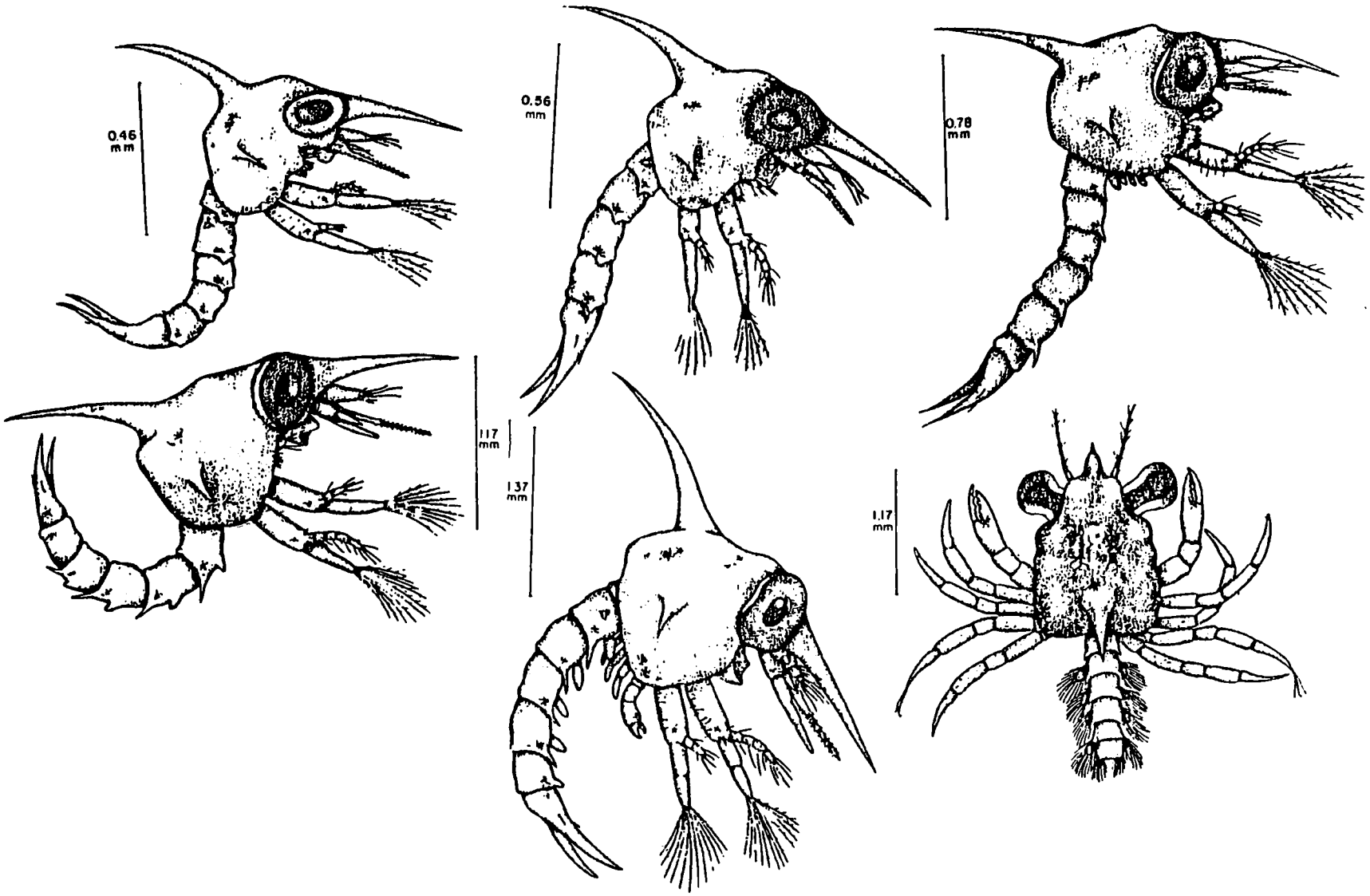


Figure 2. Larval stages of Cancer borealis I to V, zoeal stages; M. megalops.

## EFFECTS OF TEMPERATURE AND SALINITY ON DEVELOPMENT AND SURVIVAL

### Optima and limits

The temperature and salinity requirements for complete development of each species to the first crab or juvenile stage were determined by culturing larvae in different combinations of temperature and salinity (Table 2). Larvae of C. borealis completed the development only at 20 C in 30 o/oo (Fig. 3). Survival at these conditions was low (8.9%). In contrast, the larvae of C. irroratus completed development in salinities from 25-35 o/oo at 10 and 20 C, and 20-35 o/oo salinity at 15 C (Fig. 3 and 4). The highest survival to the first crab stage was observed in 30 o/oo salinity at 15 C. Ovigerous C. irroratus are found in Narragansett Bay from November to early July (Hilman, 1964; Jones, 1973; Sastry and McCarthy, 1973). Eggs of this species removed from ovigerous animals collected between November and July can be hatched when incubated in 30 o/oo salinity at 15 C. Temperature and salinity limits for complete development as well as survival under comparable conditions, varied for larvae hatched at different seasons (Fig. 5). Larvae resulting from winter hatches completed development in 30 o/oo at 10C and in 25-35 o/oo at 15 and 20 C. Larvae from spring hatches completed development in salinities from 25-30 o/oo and 10 C and 20 C, and between 20-35 o/oo at 15 C. The larvae of summer hatches failed to complete development in any salinity at 10 C, although at 20 C larvae completed development in 20-35 o/oo at 15 C and 25-35 o/oo salinity. The previous thermal history and stage of embryonic development prior to incubation affected the survival rate and limits for complete larval development.

Combined data analyzed for all seasons and represented as response surfaces (Alderdice, 1972) showed that maximum survival of C. irroratus larvae to the crab stage occur between 28-35 o/oo at temperatures between 13-17 C (Fig. 6). One hundred percent mortality of the larvae was predicted to occur below 18 and above 40 o/oo salinity, with 100% mortality beyond these limits (Fig. 6).

Larvae of H. americanus completed development to the post-larval stage in salinities between 20-35 o/oo at 15 C and 15-30 o/oo at 20 C (Fig. 3). Survival of larvae to the post-larval stage was higher in 35 o/oo at 15 C. Combined data for larvae resulting from different hatches showed that H. americanus larvae survive at a maximum rate of 80% between 20-28 o/oo salinity and 15-18 C. Development of larvae to the post-larval stage was limited by 9 and 24 C and 5 and 40% salinity with 100% mortality (Fig. 6).

Larvae of P. longicarpus completed development over a wider temperature and salinity range than larvae of any other species. The post-larval stage was reached in salinities between 15-35 o/oo at 10 and 15 C, 20-30 o/oo at 20 C and 25-30 o/oo at 25 C (Fig. 3). Survival of larvae of this species was uniformly high in all salinities from 15-35 o/oo at 20 and 25 C (Fig. 4). No single optimal combination of temperature and salinity for survival could be identified for this widely tolerant species (Fig. 6). Complete development of R. harrisii larvae was observed in 20-35 o/oo at 25 C and 15-30 o/oo at 30 C (Fig. 3). Survival of larvae to the first crab stage was highest in 25 o/oo at 25 C. Larvae of R. harrisii tolerated the highest temperatures to complete development of any of the species studied (Fig. 4).

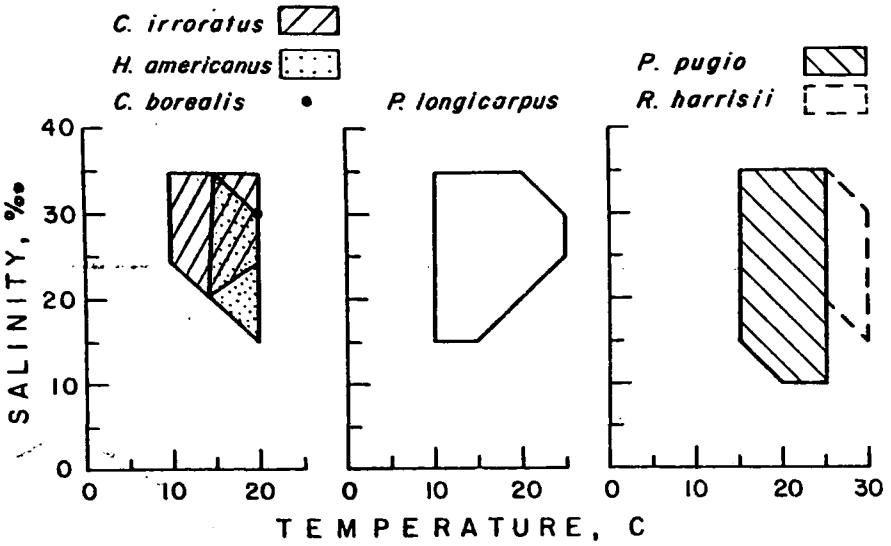


Fig. 3. Temperature and salinity limits for complete larval development of six species of crustaceans from Narragansett Bay and vicinity.

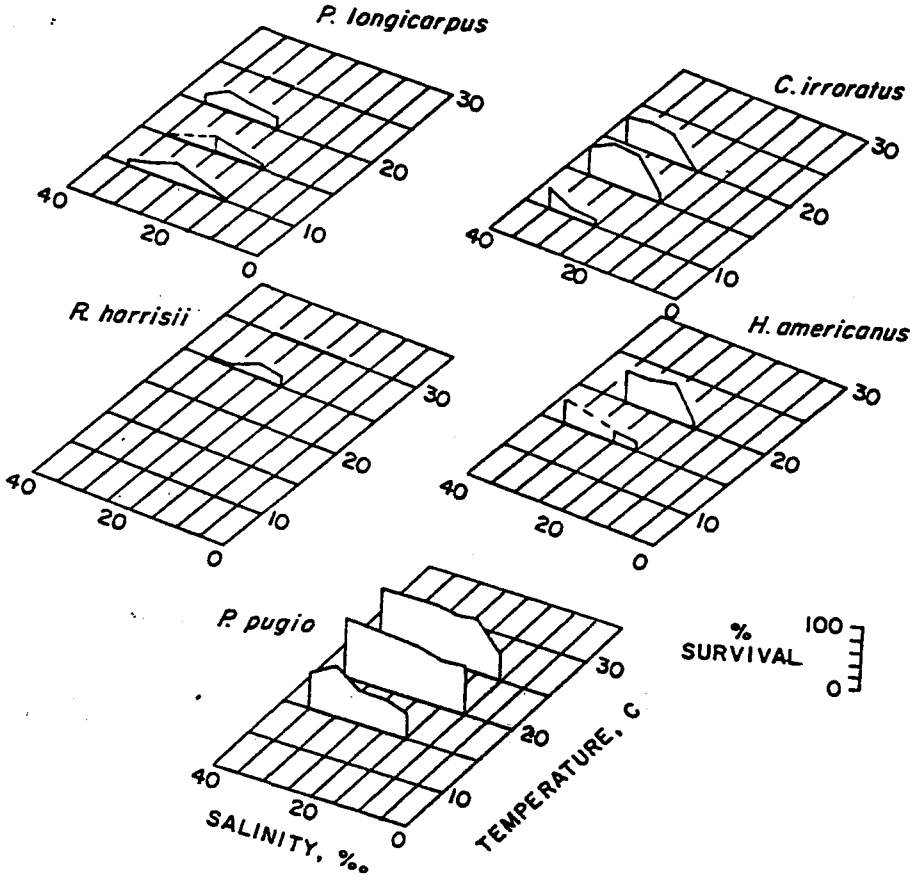


Fig. 4. Percentage survival of larvae to the post-larval stages for five species of crustaceans reared in different combinations of salinity and temperature.

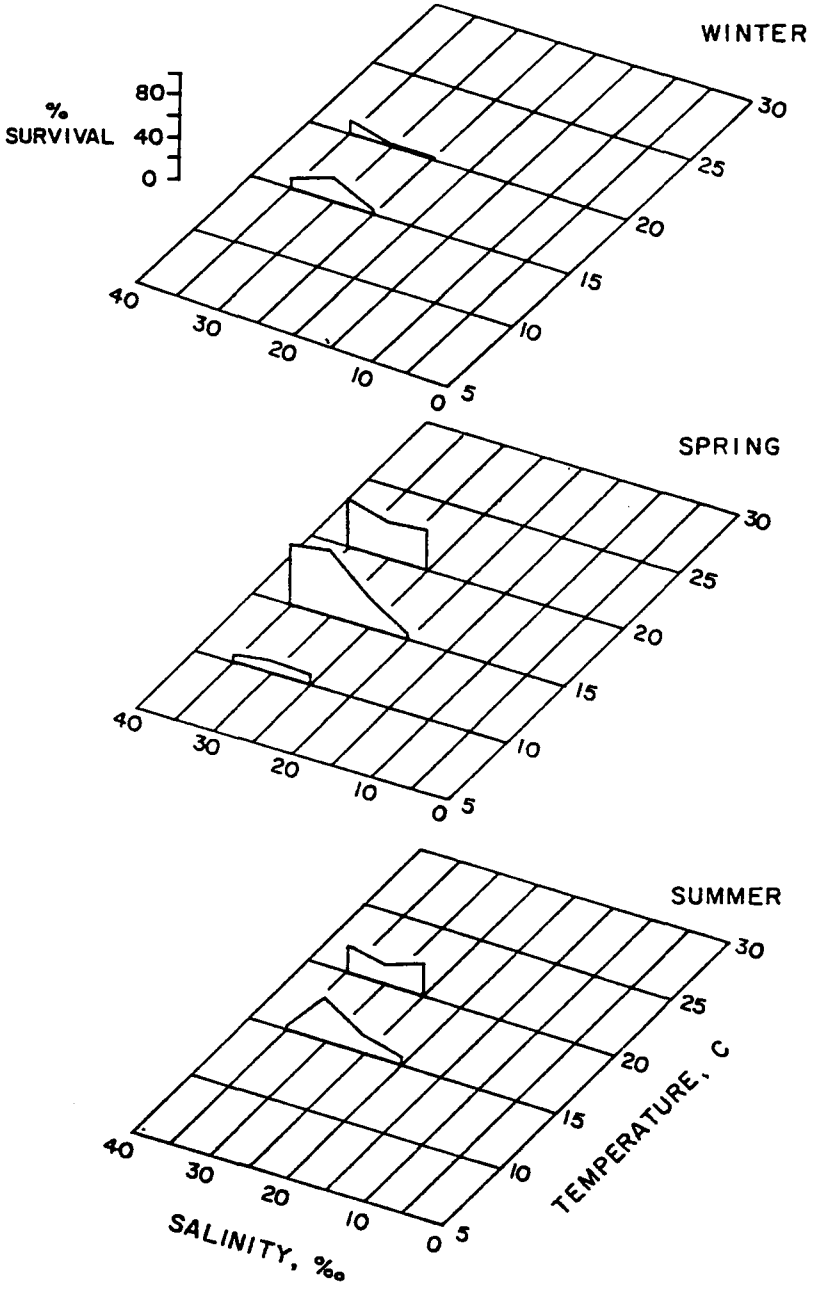


Fig. 5. Survival of C. irroratus larvae to the post-larval stage in different combinations of salinity and temperature. The larvae resulted from eggs hatched in 30 o/oo salinity at 15°C in different seasons.

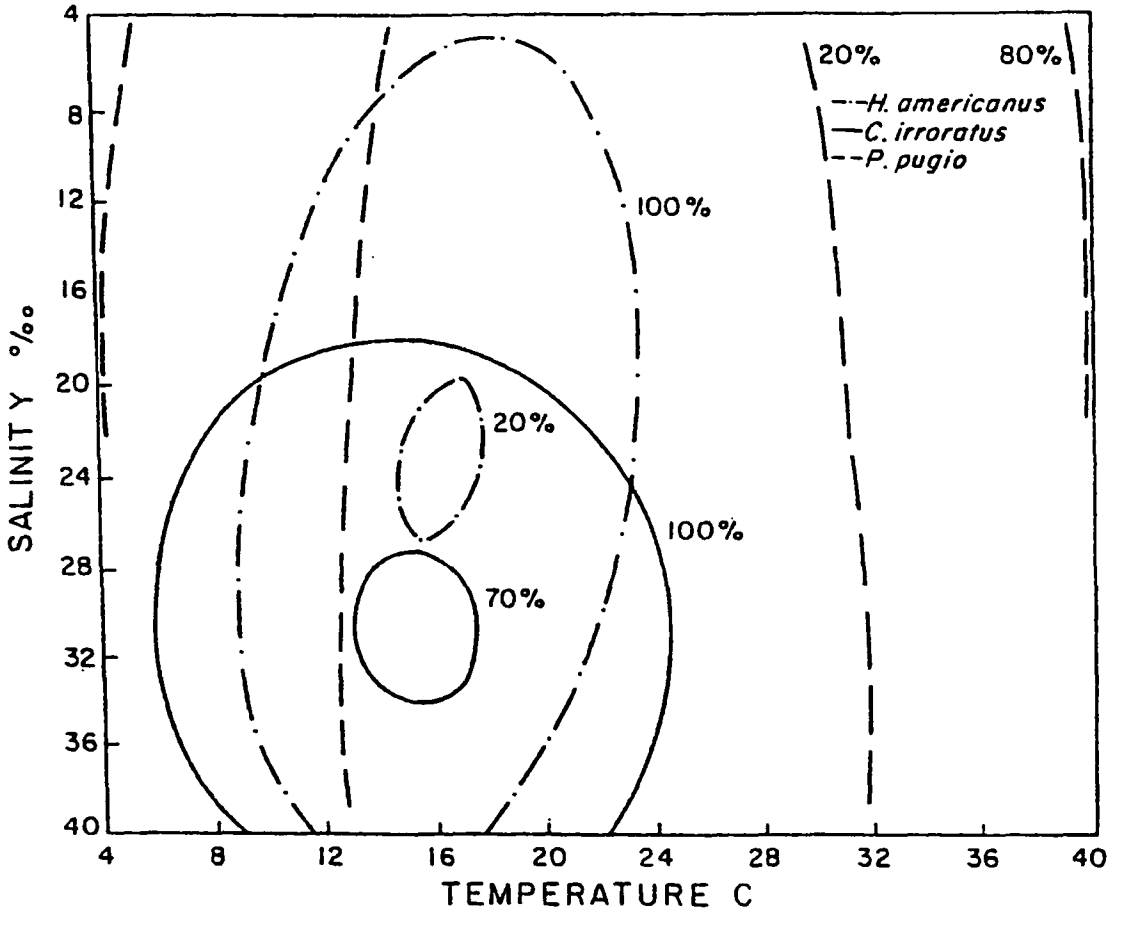


Fig. 6. Isopleths of mortality of larvae to the post-larval stage for primarily estuarine and coastal crustaceans in relation to temperature-salinity conditions.

Duration of larval development

Temperature significantly affected the duration of larval development of C. irroratus, H. americanus and P. pugio (Fig 7). In all these species, the duration for complete larval development decreased with the increase of temperature. In comparison, the developmental rate of P. longicarpus was less affected by temperature. The larvae of R. harrisii showed still another pattern of temperature effect on the rate of development, developing at a slower rate at 30 C compared to those at 25 C. The duration of larval development of all the species is affected by the temperature, but the rates vary interspecifically (Fig. 7 and Table 4).

The effect of temperature on the rate of development of each larval stage of C. irroratus was determined to examine whether successive larval stages in the development of a species are differentially affected by the temperature. The first zoeal stage of C. irroratus developed at a slower rate than the succeeding zoeal stages (Fig. 8 and Table 5). The rate of development of the zoeal stages decreased significantly at temperatures below 10 C; developmental rate remained about the same at temperatures above 25 C. The duration of megalops stage is much longer than any of the preceeding zoeal stages. The duration of the megalops stage also became significantly increased below 15 C and decreased slightly above 20 C (Fig. 8).

In contrast, salinity had much less effect on the rate of development than temperature (Fig. 9 and Table 4). The duration of development of each species was different at comparable salinities. Contrasting salinity conditions within the range of tolerances did not significantly affect the rate of development of P. longicarpus and P. pugio. Larvae of R. harrisii developed at about the same rate in salinities between 30 and 35 o/oo and at slightly slower rate in 25, 30 or 35 o/oo salinity. In contrast, the larvae of H. americanus developed at a slower rate in salinities above and below 25 o/oo.

Variation in geographically separated populations

All the species selected for this study are distributed over a wide geographical range (Table 1). Information on the duration of complete development and survival of larvae to the post-larval stage of geographically separated populations are not available for most species. Data are available for R. harrisii and P. pugio populations from North Carolina (Costlow, et al., 1966; Broad, 1957) and are compared with Rhode Island populations cultured under somewhat similar conditions (Table 6). Rhode Island larvae of R. harrisii took longer to complete development and their survival was much lower compared to those from North Carolina. In comparison, survival of P. pugio larvae from Rhode Island was higher (91.8%) compared to those from North Carolina (65%). Larval development of the Rhode Island population was completed between 18-31 days compared to 17-21 days required for the North Carolina population (Table 6). There is an indication that the duration of H. americanus larval development also varies for geographically separated populations (Table 7).

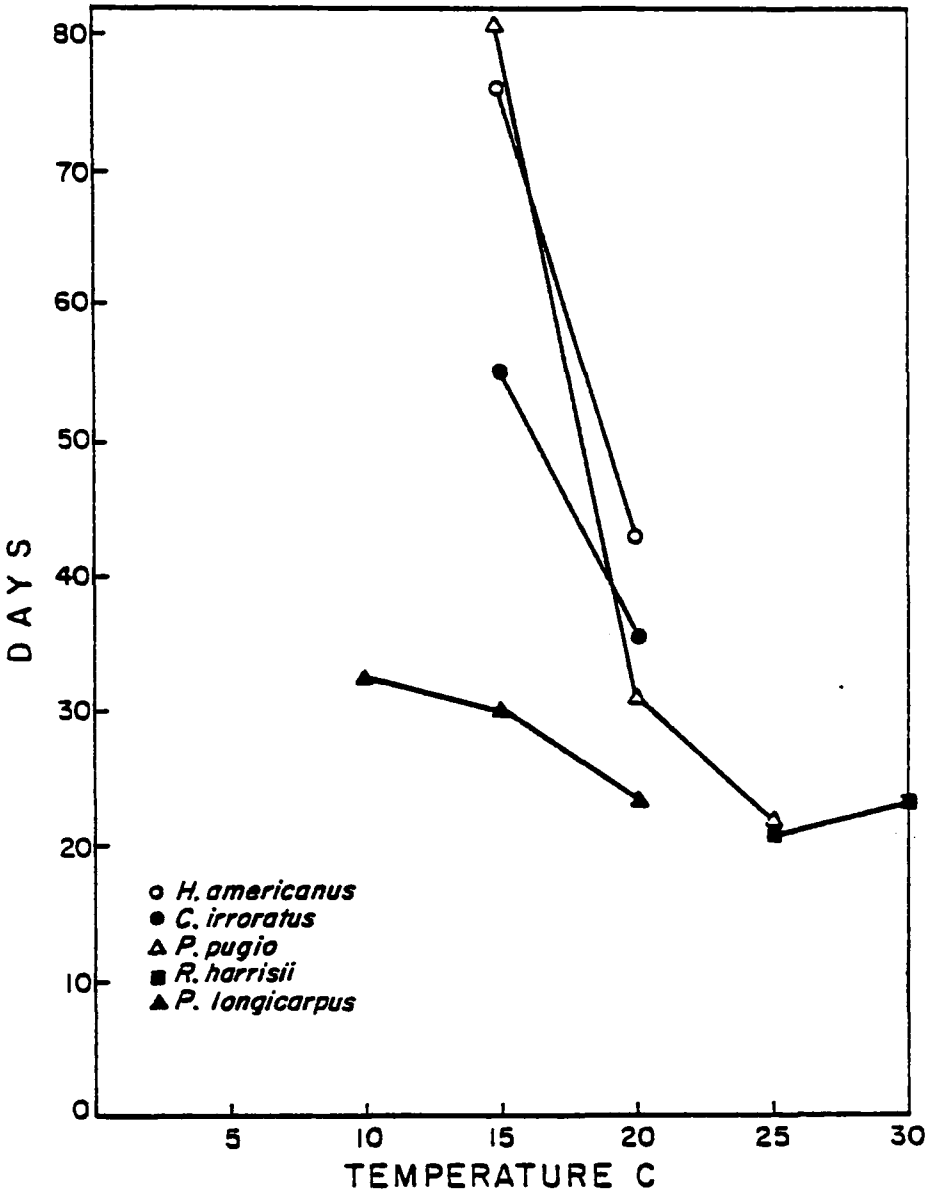


Fig. 7. Effect of temperature on the duration of larval development of primarily estuarine and coastal crustaceans.

TABLE 4. MEAN (± SD) DAYS FOR COMPLETE DEVELOPMENT TO POST-LARVAL STAGE IN VARIOUS COMBINATIONS OF TEMPERATURE AND SALINITY FOR FIVE SPECIES OF CRUSTACEANS

| Species | Temp C | Salinity | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
| Palaemonetes pugio | 15 | 101 ± 3.0 | 96.5± 11.68 | 84.46± 6.87 | 86.3± 6.31 | 81.37± 18.34 | 94.72± 15.03 | - |
| | 20 | 34.1± 3.2 | 34.6± 3.3 | 31.66± 2.28 | 32.09± 2.21 | 32.06± 2.21 | 33.27± 2.87 | - |
| | 25 | 26.32± 2.7 | 30.07± 3.66 | 22.64± 2.52 | 23.91± 3.07 | 23.56± 3.04 | 26.19± 4.70 | - |
| Paugurs longicarpus | 10 | 46.5 | 44.0 | 35.5 | 41.0 | 38.5 | | |
| | 15 | 39.0± 3.46 | 31.6± 3.81 | 32.0± 4.12 | ? | ? | | |
| | 20 | - | 31.6± 5.06 | 29.8± 4.02 | 26.3± 2.81 | 25.3± 2.73 | | |
| Rhithropanopeus harrisii | 25 | - | - | 36 | 24 | 22 | 22 | |
| | 30 | - | - | 24 | 26 | 27 | - | |
| H. americanus | 15 | - | - | 79.67± 7.02 | 97.0± 4.24 | ? | 76.0± 2.77 | |
| | 20 | - | 55 | 53.7± 8.27 | 42.27± 7.89 | 46.36± 7.28 | - | |
| C. irroratus | 15 | | - | 48.33± 3.51 | 56.39± 5.13 | 55.73± 4.24 | 60.26± 7.42 | |
| | 20 | - | - | - | 40.02± 9.94 | 36.03± 3.83 | 38.19± 6.69 | |

TABLE 5. EQUATIONS DESCRIBING THE DEVELOPMENT TIME FOR LARVAL STAGES OF CANCER IRRORATUS AT DIFFERENT CONSTANT TEMPERATURES (FROM SASTRY, 1976).

| Larval stage* | Equation |
|---|---|
| I | $D = 500/(T + 1.53)^{1.44}$ |
| II | $D = 498/(T + 1.72)^{1.53}$ |
| III | $D = 500/(T + 1.63)^{1.53}$ |
| IV | $D = 500/(T + 6.6)^{1.40}$ |
| V | $D = 999/(T + 2.06)^{1.70}$ |
| Megalops | $D = 500/(T - 3.19)^{1.29}$ |

*Stages I to V are zoeal stages.

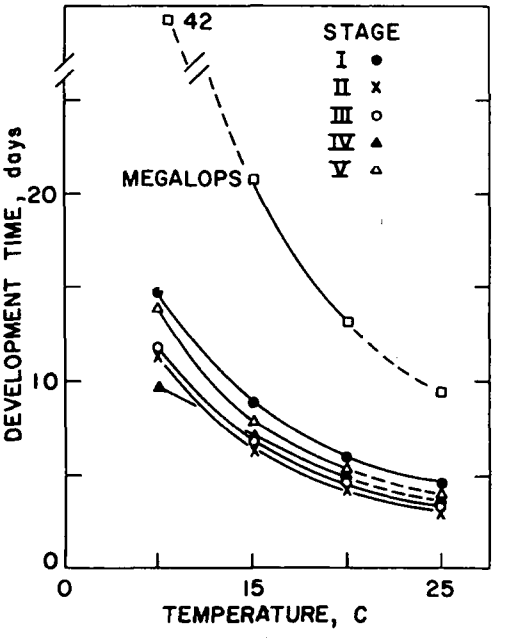


Figure 8. Effect of temperature on development time for larval stages of C. irroratus. Dashed lines on the curves represent extrapolated valu

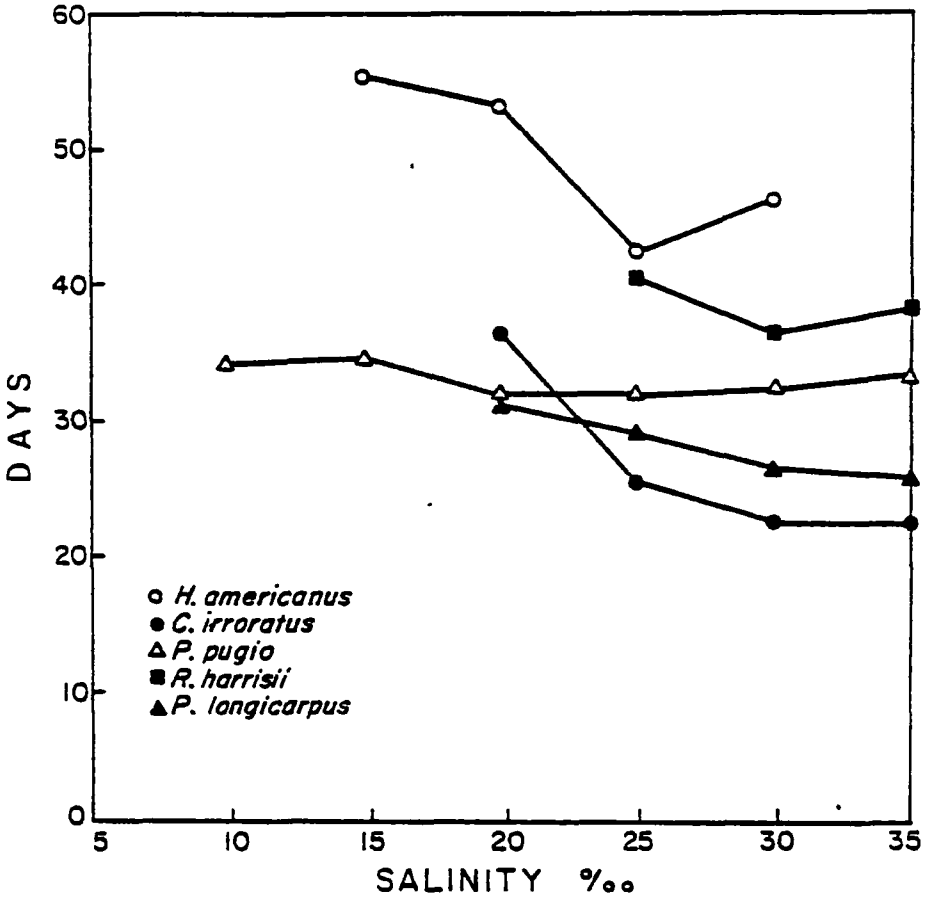


Figure 9. Effect of salinity on the duration of larval development of primarily estuarine and coastal crustaceans.

TABLE 6. VARIATION IN THE SURVIVAL AND DURATION FOR COMPLETE DEVELOPMENT OF GEOGRAPHICALLY SEPARATED POPULATIONS OF THREE SPECIES OF CRUSTACEANS.

| Species | Rhode Island | | | North Carolina* | | |
|---|---|---|---|---|---|---|
| | T (C), S (o/oo) | % Survival | Duration Days | T (C), S (o/oo) | % Survival | Duration Days |
| *Panopeus herbatii* | 20 C<br>32 o/oo | 52 | 30-38 | 20 C<br>31.1 o/oo | 0 | - |
| | 25 C<br>32 o/oo | 48 | 20-32 | 25 C<br>31.1 o/oo | 3 | 33-35 |
| *Rhithropanopeus harrisii* | 25 C<br>25 o/oo | 20.7 | 20-30 | 25 C<br>25 o/oo | 83 | 15-23 |
| | 25 C<br>35 o/oo | 2.0 | 22 | 25 C<br>35 o/oo | 23 | 16-21 |
| | 30 C<br>15 o/oo | 1.89 | 23-24 | 30 C<br>15 o/oo | 14 | 15-22 |
| | 30 C<br>25 o/oo | 4.08 | 23-30 | 30 C<br>25 o/oo | 60 | 11-15 |
| *Palaemonetes pugio* | 25 C<br>30 o/oo | 91.8 | 18-31 | 25-27 C | 65 | 17-21 |

* Data from Costlow, Bookhout and Monroe (1966); Broad (1957)

TABLE 7. DURATION (DAYS) FOR DEVELOPMENT TO THIRD AND FOURTH LARVAL STAGE OF H. AMERICANUS FROM DIFFERENT GEOGRAPHICAL REGIONS (THE FOOD FOR LARVAE AND CULTURE CONDITIONS ARE SOMEWHAT DIFFERENT).

| Geographical region | Culture temperature C | Days to 4th stage | Days to 5th stage | Reference |
|---|---|---|---|---|
| St. Andrew's | 6-7 | 120 | | |
| Canada | 10 | 54.5 | 100 | |
| | 14 | 26.5 | 50 | Templem an (1936) |
| | 19 | 13.5 | 28 | |
| | 24 | 10.5 | | |
| Nova Scotia & | 15 | 25 | | Sherman and Lewis |
| Maine | 19-20 | 15 | | 1967; Scaratt, 1968 |
| Martha's vineyard, Mass. | 19-20 | 13.6 | | Hughes and Mattheissen, 1962 |
| Rhode Island | 10 | 71 | 109 | |
| | 15 | 29 | 76 | This study |
| | 20 | 23 | 46 | |

Effects of constant and daily cyclic temperatures

The effects of daily cyclic and comparable constant temperatures on the duration of larval development and survival were determined for the larvae of C. irroratus and P. pugio.

Larvae of C. irroratus cultured at 10 to 20 and 25 C daily cyclic temperatures experienced greater survival than those at comparable constant temperatures (Fig. 10 and Tables 8 and 9). Survival of larvae was less at 12.5-17.5 C cycle than that at the 10-20 C cycle. Larvae cultured at the 17.5-22.5 C cycle developed only to the megalops stage. Duration of the zoeal stages at both 12.5-17.5 C and 10-20 C daily cycles decreased and megalops duration increased compared to that at constant 15 C (Fig. 11 and Table 10). The duration of the megalops stage in-

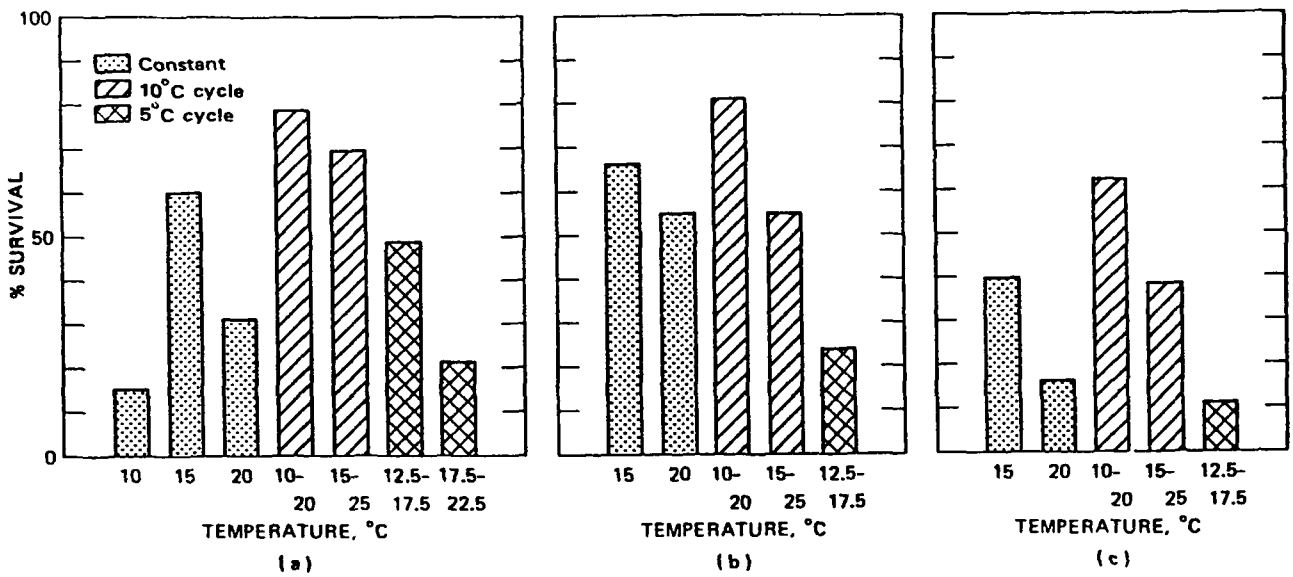


Figure 10. Percentage survival of C. irroratus larvae at constant and cyclic temperatures. (a) Hatch to megalops, (b) Megalops to crab, (c) Hatch to crab.

TABLE 8. SURVIVAL OF C. IRRORATUS LARVAL STAGES AT CONSTANT AND CYCLIC TEMPERATURES.

| Larval stage* | Survival at constant temperature, % | | | | Survival at cyclic temperatures, % | | | |
|---|---|---|---|---|---|---|---|---|
| | 10°C | 15°C | 20°C | 25°C | 10–20°C | 15–25°C | 12.5–17.5°C | 17.5–22.5°C |
| I | 54 | 85 | 76 | 39 | 88 | 87 | 82 | 76 |
| II | 37 | 78 | 68 | 17 | 86 | 83 | 72 | 60 |
| III | 32 | 72 | 58 | 6 | 84 | 78 | 66 | 40 |
| IV | 20 | 66 | 47 | 0 | 79 | 75 | 58 | 27 |
| V | 15 | 60 | 31 | 0 | 78 | 71 | 49 | 20 |
| Megalops | 0 | 40 | 17 | 0 | 63 | 39 | 12 | 0 |

*Stages I to V are zoeal stages.

TABLE 9. STATISTICAL ANALYSIS TESTING THE SIGNIFICANCE OF DIFFERENCES BETWEEN SURVIVAL OF LARVAL STAGES OF C. IRRORATUS CULTURED AT CONSTANT AND CYCLIC TEMPERATURES.

| Temperature, °C | Larval stage† | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | Megalops |
| 15 vs. 10–20 | – | – | – | – | + | + |
| 20 vs. 15–25 | – | + | + | + | + | + |
| 15 vs. 12.5–17.5 | – | – | – | – | – | + |
| 20 vs. 17.5–22.5 | – | – | + | + | – | + |
| 10–20 vs. 15–25 | – | – | – | – | – | + |
| 12.5–17.5 vs. 17.5–22.5 | – | – | – | + | + | + |
| 10–20 vs. 12.5–17.5 | – | – | + | + | + | + |
| 15–25 vs. 17.5–22.5 | – | + | + | + | + | + |

*Minus (–) means not significant; plus (+) means $p < 0.05$ (Pearson and Hartley, 1954).

†Stages I to V are zoeal stages.



TABLE 10. MEAN DAYS AND PROPORTION OF TOTAL DEVELOPMENT TIME (HATCH TO CRABS) FOR EACH LARVAL STAGE OF C. IRRORATUS AT CONSTANT AND CYCLIC TEMPERATURES.

| Larval stage† | Constant temperatures | | | | | | Cyclic temperatures | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10°C | 15°C | | 20°C | | 25°C | 10-20°C | | 15-25°C | | 12.5-17.5°C | | 17.5-22.5°C |
| | Days | Days | % | Days | % | Days | Days | % | Days | % | Days | % | Days |
| I | 15.4 ± 6.8 | 6.5 ± 1.4 | 11.6 | 5.2 ± 1.3 | 14.4 | 4.2 ± 2.4 | 5.1 ± 1.0 | 9.1 | 3.6 ± 1.2 | 6.2 | 5.6 ± 2.7 | 10.8 | 6.9 ± 4.7 |
| II | 26.0 ± 6.6 | 12.9 ± 2.3 | 11.4 | 9.1 ± 2.0 | 10.8 | 8.8 ± 0.41 | 11.4 ± 3.5 | 11.3 | 7.9 ± 2.0 | 7.4 | 11.6 ± 3.6 | 11.7 | 13.3 ± 5.1 |
| III | 38.7 ± 10.3 | 19.4 ± 2.7 | 11.6 | 13.2 ± 2.5 | 11.3 | 13.0 | 17.9 ± 3.5 | 11.6 | 13.6 ± 2.8 | 9.8 | 18.4 ± 3.4 | 13.2 | 18.5 ± 6.4 |
| IV | 41.4 ± 12.6 | 26.0 ± 2.8 | 11.8 | 17.8 ± 1.7 | 12.7 | | 23.5 ± 3.5 | 10.0 | 19.1 ± 3.5 | 9.5 | 23.9 ± 3.0 | 10.7 | 24.8 ± 3.3 |
| V | 61.3 ± 4.4 | 35.0 ± 3.2 | 16.1 | 24.2 ± 2.0 | 17.7 | | 30.1 ± 2.5 | 11.8 | 24.3 ± 3.9 | 8.9 | 28.9 ± 2.4 | 9.7 | 30.4 ± 3.7 |
| Megalops | | 55.7 ± 4.2 | 37.5 | 36.0 ± 3.8 | 32.8 | | 56.0 ± 5.5 | 46.3 | 57.9 ± 11.3 | 58.0 | 51.5 ± 2.4 | 43.9 | |

*Weighted mean.

†Stages I to V are zoeal stages.

creased considerably at the 15-25 C cycle compared to constant 20 C. The megalops was the most thermally sensitive stage in the larval development of C. irroratus.

Larval survival from eggs of C. irroratus hatched under daily cyclic temperatures and reared under the same temperature cycle showed an increase in the survival to the crab stage, compared to larvae hatched at constant temperature and reared under daily cyclic regime (Fig. 12.) Survival increased by 9% for the 10-20 C cycle, 7% for the 15-25 C cycle and 1.5% for the 12.5-17.5 C cycle. Larval development time whether eggs hatched under cyclic or constant temperature, was not significantly different for the 10-20 and 12.5-17.5 cycles. However, for the 15-25 C cycle, the development time of larvae hatched under cyclic temperatures was less than that for larvae hatched under constant conditions (Fig. 12).

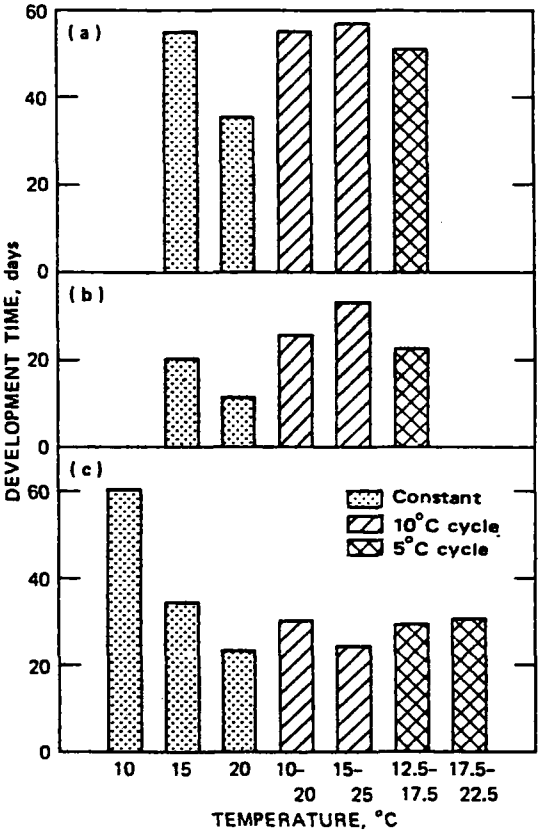


Figure 11. Duration of larval development of C. irroratus at constant and cyclic temperatures. (a) Hatch to crab. (b) Megalops. (c) Hatch to megalops.

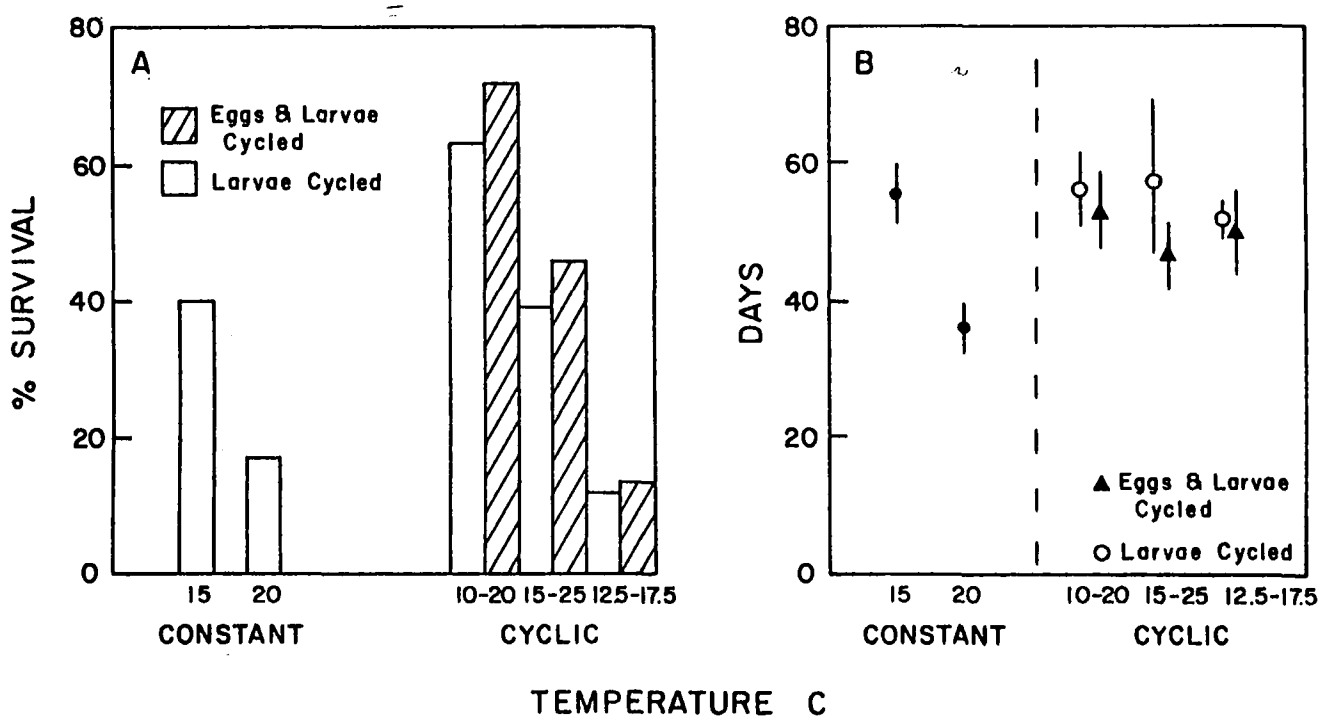


Figure 12. Survival of C. irroratus larvae to the post-larval stage under constant and comparable cyclic temperature regimes (A). The duration of larval development under constant and comparable cyclic temperature regimes (B).

Larvae of P. pugio cultured at 12.5 to 17.5 and 10-20 C daily cyclic temperatures survived to the post-larval stage slightly better than those at comparable constant 15 C (Fig. 13). No significant differences in the rate of survival to the post-larval stage were observed at 17.5-22.5 and 15-25 C daily cycles and at comparable constant 20 C. Duration of development to the post-larval stage was slightly longer at constant 15 C compared to that at 10-20 or 12.5-17.5 C daily cycles. No significant differences were observed in the duration of development at constant 20 C and 15-25 or 17.5-22.5 C daily cyclic temperatures (Fig. 14).

Mean weight of the developmental stages of C. irroratus increased progressively from first zoeal stage to the crab stage. There were no significant differences in the weight of larval stages cultured at 10-20 C daily cycle and comparable constant temperature ($p<0.01$). Mean weight of the larval stages of P. pugio also increased progressively with development.

## METABOLIC-TEMPERATURE RESPONSES

### Larvae Cultured at Constant Temperatures

Larvae cultured at a temperature and salinity producing highest survival to the post-larval stage (C. irroratus,15C-30 o/oo; C. borealis, 20 C-30 o/oo; P. pugio, 20 C-30 o/oo) showed both inter and intraspecific variation in their metabolic responses to temperature. The first stage larvae of C. irroratus were metabolically active over a temperature range of 5-25 C (Fig. 15), with a $Q_{10}$ close to 2. For successive stages, the temperature range for depression ($Q_{10}<1$) of metabolic rate was about the same, but differences in temperature ranges for sensitivity ($Q_{10}>2$) and compensation ($1<Q_{10}<2$) were observed. The temperature range for sensitivitv in the second and third zoeal stage was from 5-15, but this shifted to 10-15C in the fourth and fifth stages. The temperature range for compensation has also narrowed from 10-20 C in the second and third stage to 15-20 C in the fourth and fifth stages. The megalops showed no compensation over the 10-20 C range. The metabolic rate of all the stages was depressed between 20-25 C.

The larval stage of C. borealis showed a distinctly different metabolic response to temperature. Larvae of this species were metabolically active over the entire temperature range of 5-25 C and there was no depression of metabolic rate at warmer temperatures (Fig. 16 and 17). The first and second stage zoeae showed compensation over a temperature range of 5-20 C and 5-15 C, respectively. Sensitivity was observed at warmer temperatures. The third stage showed no compensation over the entire temperature range of 5-25 C. Metabolic-temperature patterns of the fourth, fifth and megalops stage are reversed from those of the first two with sensitivity to colder temperatures and compensation to warmer temperatures.

The metabolic response patterns of P. pugio to temperature are shown as $Q_{10}$ values (Fig. 17). Larvae of this species were metabolically active over a wider temperature range and at warmer temperatures than the larvae of the two sublittoral species. The first and second larval stages of P. pugio compensated between 15-20 C and 20-25 C, respectively. The temperature range

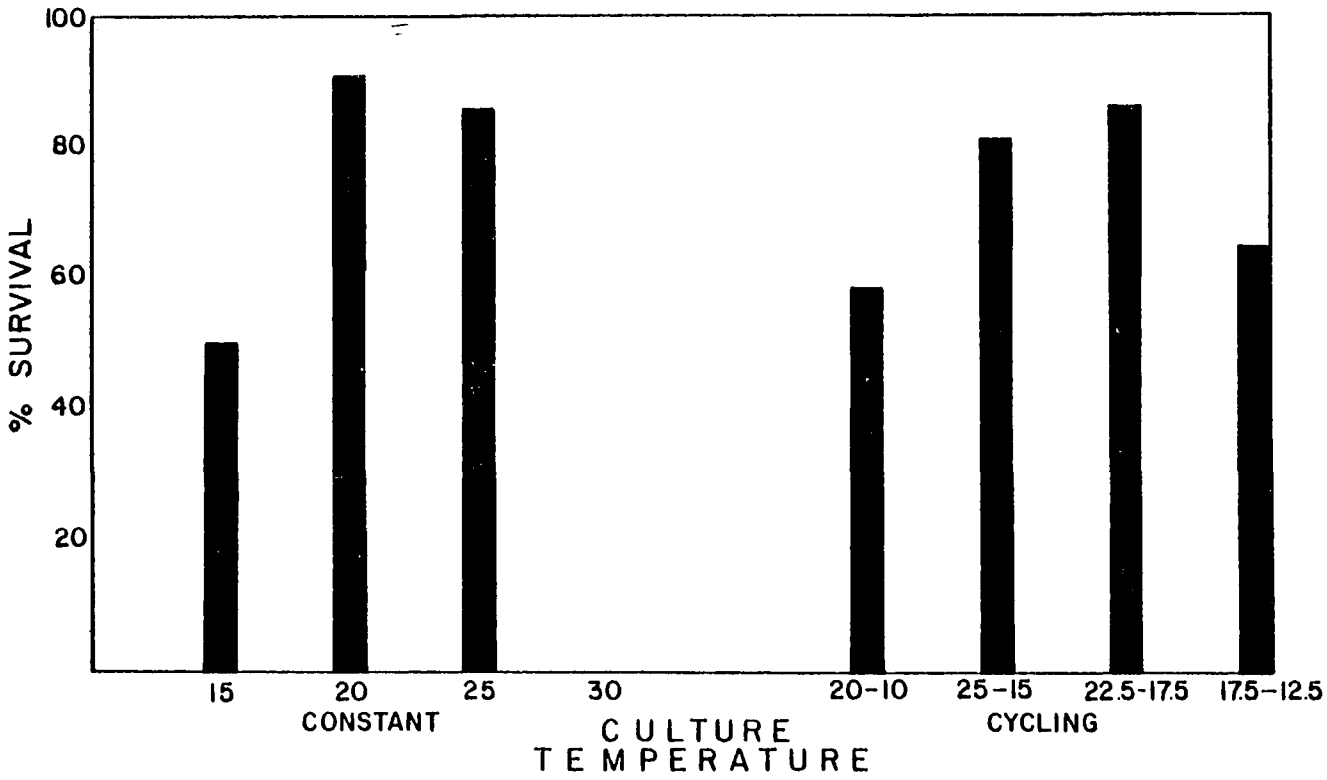


Figure 13. Percentage survival of Palaemonetes pugio at constant and daily cyclic temperatures.

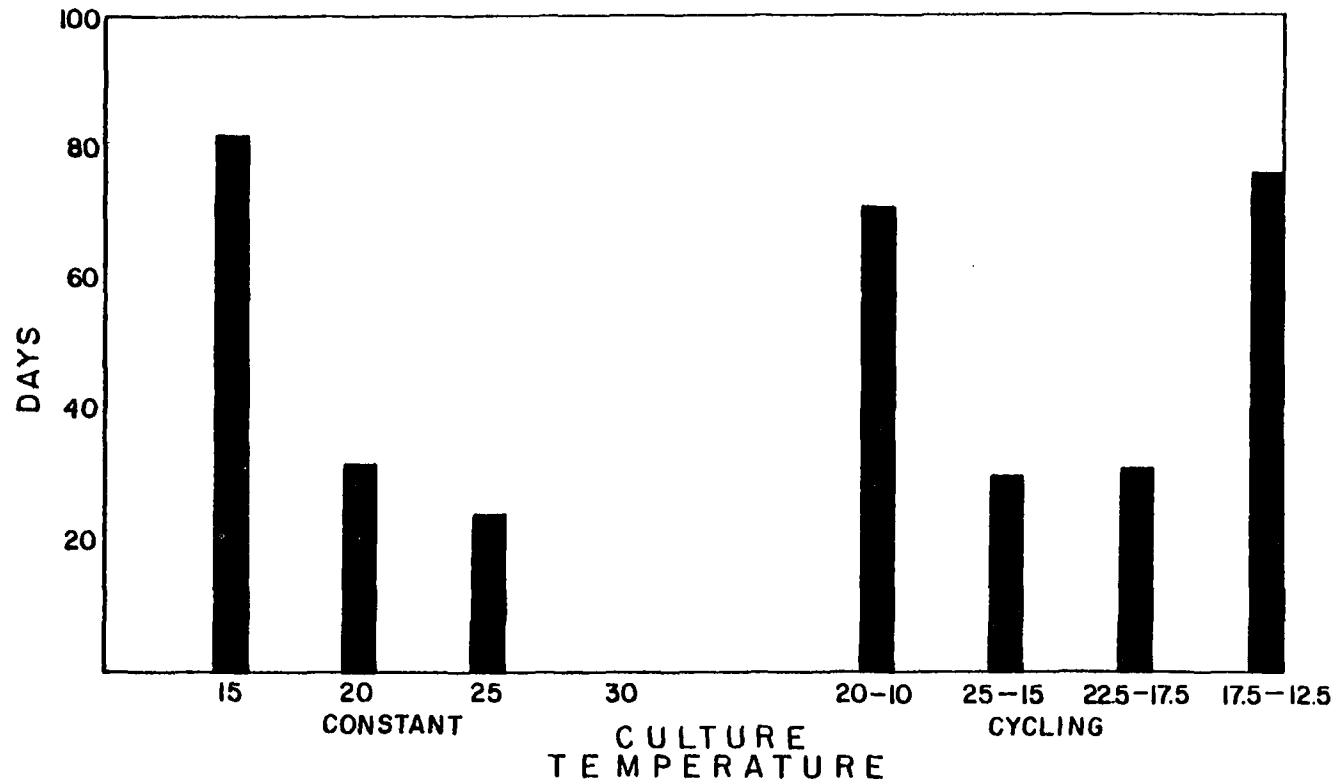


Figure 14. Duration of larval development of Palaemonetes pugio at constant and daily cyclic temperatures.

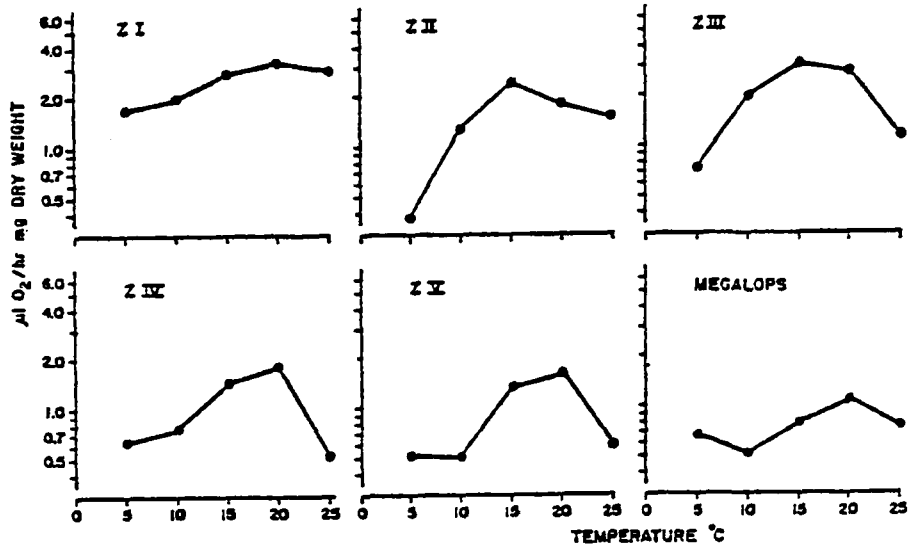


Figure 15. Metabolic-temperature response of different larval stages of Cancer irroratus; ZI to ZV zoeal stages.

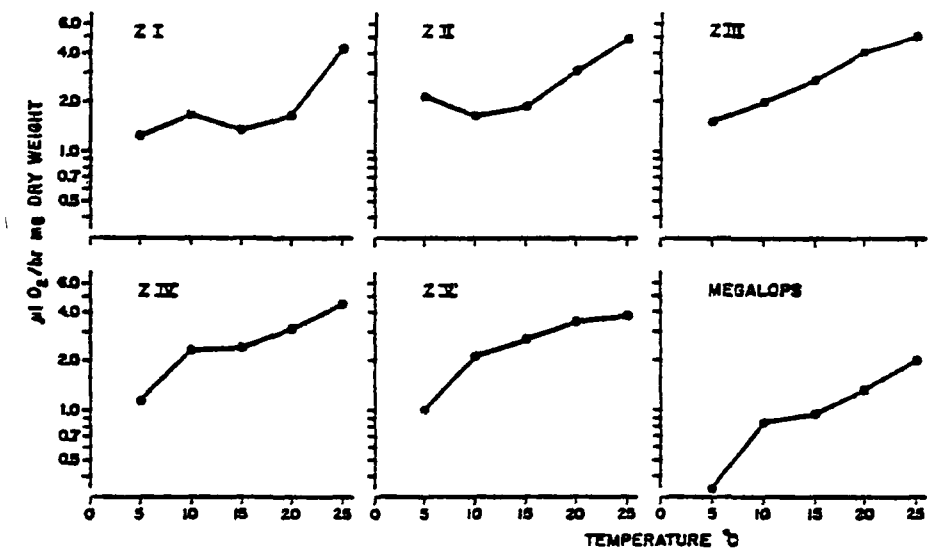


Figure 16. Metabolic-temperature response of different larval stages of Cancer borealis; ZI to ZV zoeal stages.

for metabolic compensation was much wider for the third through seventh stage larvae. In the fourth stage compensation extended to warmer temperatures than in the earlier stages. The fourth stage showed no compensation from 10-25 C and compensated from 25-35 C. The fifth stage was the reverse. In the eighth stage, compensation occurred between 15-20 C and 25-30 C. At the extreme warm temperatures of 30-35 C, the metabolic rate of the third, sixth, and eighth stages was depressed.

Larvae Cultured at Daily Cyclic Temperatures

Oxygen consumption rates of the developmental stages of C. irroratus cultured at the constant 15 C and 10-20 C daily cyclic temperatures were different at some of the test temperatures. These differences in rate were significant ($p<0.01$) for only first and second zoeal stages and the first crab stage at 15 C. At the 20 and 25 C test temperatures, almost all stages from the cyclic regime respired faster than those from constant culture temperature. No obvious correlation of the variation of oxygen consumption rates with the stage of development was evident.

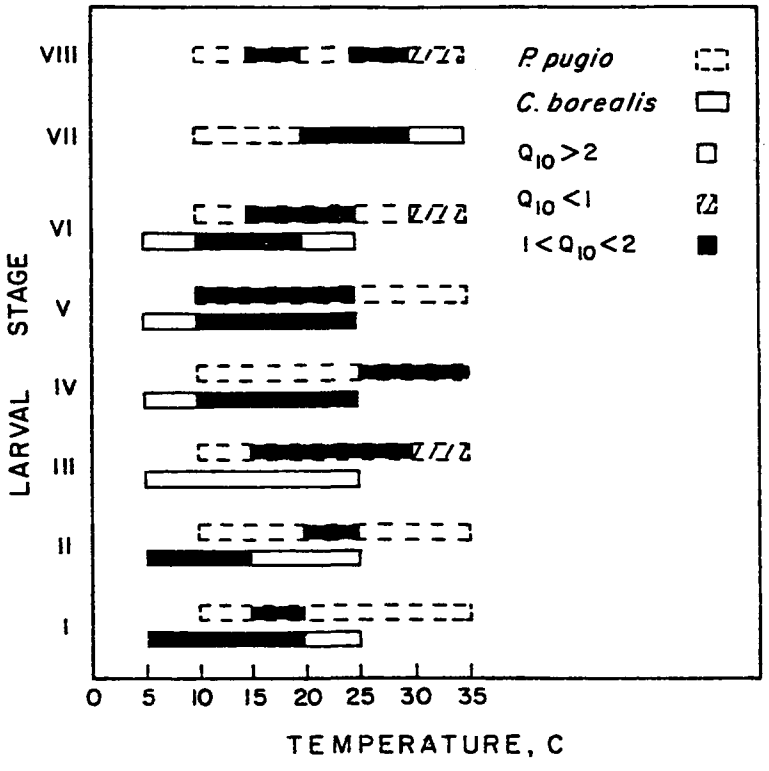


Figure 17. Metabolic-temperature responses (as $Q_{10}$ values) of larval stages of C. borealis and P. pugio cultured at constant 20°C and 30 o/oo salinity.

Larvae of C. irroratus cultured at 10-20 C daily cyclic temperatures had different metabolic-temperature response patterns from those larvae from constant 15 C as reflected in the temperature range of sensitivity ($Q_{10}>2$), compensation ($1<Q_{10}<2$), and depression ($Q_{10}<1$) of metabolic rate (Fig. 18).

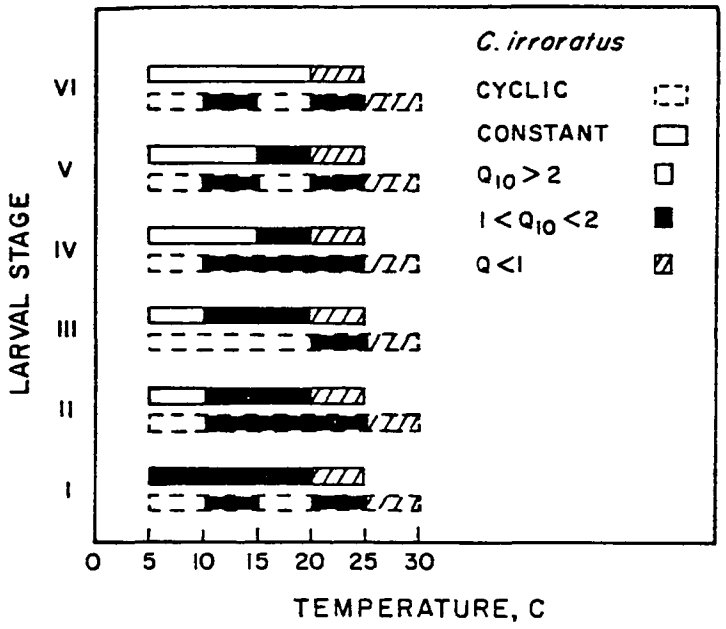


Figure 18. Metabolic-temperature responses (as $Q_{10}$ values) of larval stages of C. irroratus cultured in 30 o/oo at constant 15°C and cyclic 10°-20°C.

Larvae experiencing the daily cyclic temperature regime exhibited an extension of the temperature range for compensation, with a shift towards the higher temperatures. Oxygen consumption rate for larvae cultured with cyclic temperatures was higher than those from constant temperatures at warmer test temperatures. The temperature range for depression of metabolic rate shifted from 20-25 C range for constant larvae to 25-30 C range for the cyclic larvae.

Larval stages of the American lobster, H. americanus, cultured at 15-25C and 17.5-22.5 C daily cyclic temperatures and constant 20 C also showed inter and intra-stage variation in their metabolic-temperature response patterns (Fig. 19). Larvae cultured at constant 20 C showed no compensatory response of metabolic rate in the first larval stage, but compensatory response was observed between 15-25 C for the second stage, 10-15 C for the third stage and 15-25 C for the fourth stage. Metabolic rate of the first and third stage larvae was depressed between 20-30 C, compared to the 25-30 C range for the second and fourth stages. Culture at the daily cyclic regimes has altered the metabolic-temperature response patterns of the larval stages relative to those at constant temperature. These differences were reflected in the zones of thermal sensitivity and compensation, but the pattern of these changes were different for the larval stages.

Larval stages of the estuarine grass shrimp, P. pugio, cultured at 15-25 C daily cyclic and 20 C constant temperatures showed both inter and intra-stage variation in their metabolic responses to temperatures. However, inter-stage variation between the two culture regimes had no constant or predictable pattern (Fig. 20). Culture at cyclic temperatures enhanced the respiration rate in some stages and reduced the rate in other stages. First stage larvae at the cyclic regime showed compensation between 10-15 C

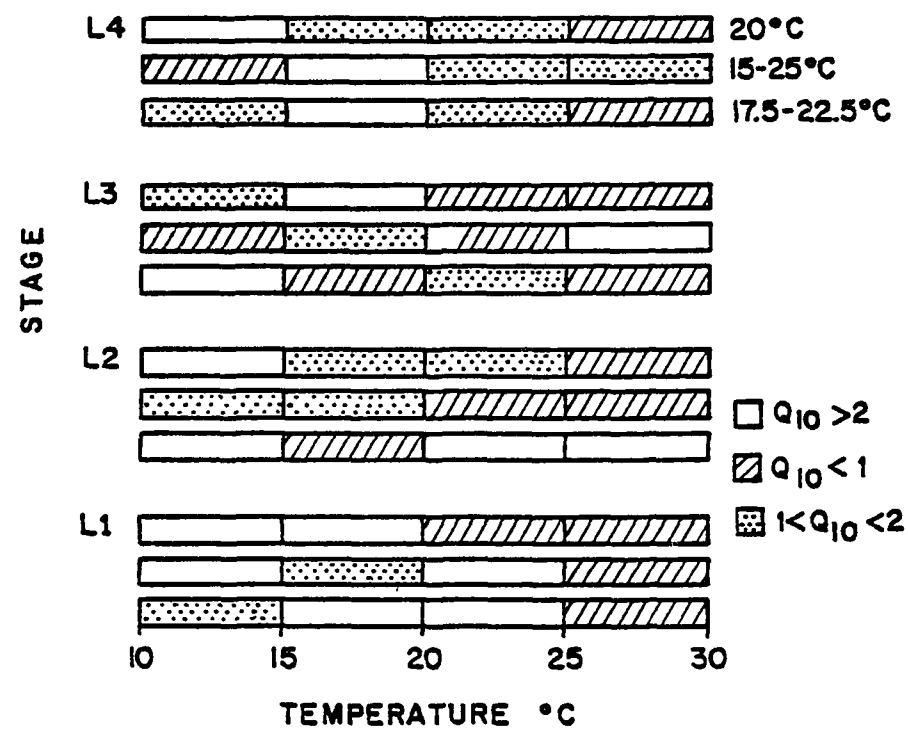


Figure 19. Metabolic-temperature responses (as $Q_{10}$ values)of larval stages of Homarus americanus cultured in 30 o/oo temperature-salinity at constant 20 C and cyclic 15-25 and 17.5-22.5 C.

and depression between 25-35 C ranges. Metabolic rate of the second stage was depressed over both the 10-15 and 30-35 C ranges, with compensation between 25-30 C. The third stage compensated between 10-15 C, with depression between 30-35 C. For the fourth stage metabolic rate was compensated between 10-15 and 25-30C and depressed between 30-35 C. The fifth stage compensated over a broader range than the earlier stages, i.e. between 20-35 C. The sixth stage compensated between 15-25 C and has depressed between 30-35C. In the seventh stage metabolic rate compensation was observed between 15-20 and 25-30 C and depresssion between 30-35 C. The eighth stage larvae compensated between 10-20 and depression occurred between 25 and 35 C. Larval stages cultured at constant 20 C showed equally variable metabolic temperature responses as those at the daily cyclic regime.

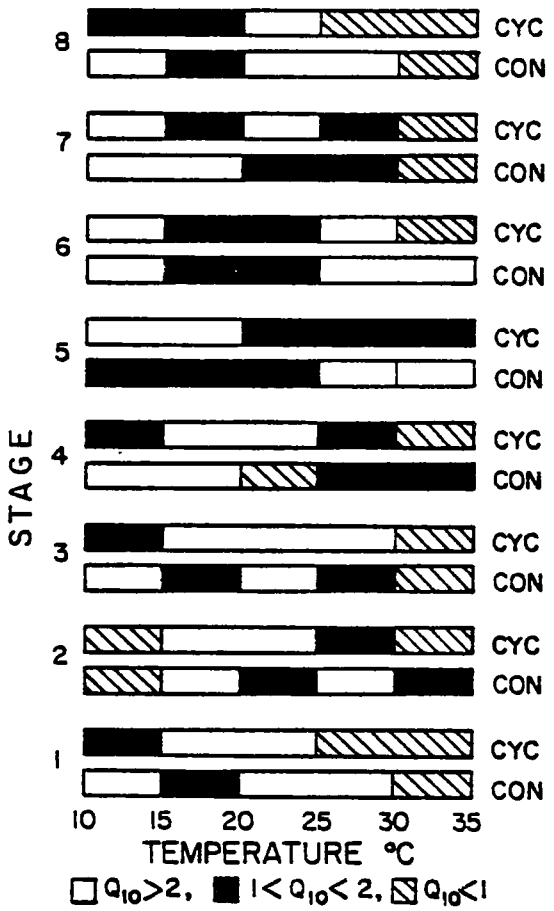


Figure 20. Metabolic-temperature responses (as $Q_{10}$ values) of larval stages of Palaemonetes pugio cultured in 30 o/oo salinity at constant 20 C and daily cyclic 15-25 and 17.5-22.5 C temperatures.

FATTY ACID METHYL ESTERS

Fatty acid methyl esters were determined for the developmental stages of C. irroratus beginning with eggs to post-larvae cultured at constant 15 C and H. americanus cultured at constant 20 C and 15-25 daily cyclic temperatures. In general, the fatty acid methyl esters in the developmental stages of both species showed minor qualitative differences, except for the presence of detectable amounts of 14:0 and 22:5 chain length fatty acids in C. irroratus (Table 11).

Fatty acids 16:0, 16:1, 18:1, 20:5 and 22:6 were present in higher concentrations than 14:0, 18:0, 20:1 and 20:4 and 22:5 in the eggs of C. irroratus. In the succeeding larval stages, the fatty acids 16:0 and 20:5 remained at a fairly constant concentration, while 22:6 decreased from a high concentration in the eggs and first zoeae to low levels in the later megalops and crab stages. Fatty acids 18:2 and 18:3 were observed in only trace amounts in eggs and first zoeae, but they increased to detectable amounts in the megalops and crab stages. The 22:5 chain length fatty acid present in detectable amount in eggs and first stage zoeae decreased to trace amounts in the megalops and crab stage.

In H. americanus cultured at constant 20 C, fatty acids 16:0 and 18:1 were present in high concentrations and 16:1, 18:0 and 20:4 in low concentrations through all the developmental stages from eggs to post-larvae. 20:5 and 22:6 chain length fatty acids were initially present in high concentrations in eggs and first larval stage then decreased progressively with development to the post-larval stage. Fatty acid chain lengths 18:2 and 18:3 in trace amounts in eggs and first larval stage increased to detectable amounts in the later stages of development, while 20:1 in high concentrations in the eggs and first stage decreased beginning with the second stage.

In H. americanus cultured with 15-25 C daily cyclic temperatures, the 16:1, 18:1 and 20:5 chain length fatty acids were present in fairly high concentrations through all stages beginning with eggs to the post-larvae. Fatty acids 16:1, 18:0 and 20:4 were in low concentrations through all the stages of development. Initially high concentrations of 22:6 fatty acid in eggs steadily decreased with the progress of development to the post-larval stage, whereas 18:2 and 18:3 were present in only trace amounts in the eggs, then increased to detectable amounts beginning with the first larval stage. Fatty acid 20:1 was present in detectable amounts in the eggs, then decreased to trace amounts beginning with the first larval stage.

A comparison of fatty acid methyl esters in H. americanus larvae cultured at constant and daily cyclic temperatures showed that certain fatty acids had a tendancy for greater unsaturation under fluctuating thermal regime in some stages of development (i.e. 16:0). The other difference observed was an increase of certain fatty acids present in only trace amounts at constant temperature to detectable amounts in those cultured under cyclic temperatures (i.e. 18:2 and 18:3). Fatty acid (i.e. 20:1), present in only detectable amounts in H. americanus cultured at constant 20 C, were only present in trace amounts in those under cyclic regime.

TABLE 11. FATTY ACID METHYL ESTERS IDENTIFIED IN DEVELOPING LARVAL STAGES OF HOMARUS AMERICANUS CULTURED AT CONSTANT 20 C AND 15 - 25 C CYCLIC TEMPERATURES

| Stage | | Chain length | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 20:4 | 20:5 | 22:6 |
| Eggs | 20 | +++ | ++ | ++ | +++ | T | T | ++ | ++ | +++ | +++ |
| | 15-25 | +++ | ++ | ++ | +++ | T | T | ++ | ++ | +++ | +++ |
| I | 20 C | +++ | ++ | ++ | +++ | T | T | ++ | ++ | +++ | +++ |
| | 15-25 | +++ | ++ | ++ | +++ | ++ | ++ | T | ++ | +++ | +++ |
| II | 20 | +++ | ++ | ++ | +++ | ++ | ++ | T | ++ | +++ | ++ |
| | 15-25 | +++ | ++ | ++ | +++ | ++ | ++ | T | ++ | +++ | ++ |
| III | 20 | +++ | ++ | ++ | +++ | ++ | ++ | T | ++ | ++ | ++ |
| | 15-25 | +++ | ++ | ++ | +++ | ++ | ++ | T | ++ | +++ | ++ |
| IV | 20 | +++ | ++ | ++ | +++ | ++ | ++ | T | ++ | ++ | ++ |
| | 15-25 | +++ | ++ | ++ | +++ | ++ | ++ | T | ++ | +++ | ++ |

+++ indicates above 10%, ++ less than 10% and T trace amounts per gram dry weight

ENZYME ACTIVITIES

The potential influence of constant vs. daily cyclic temperatures on larvae was examined by assaying the specific activities of lactate dehydrogenase, malate dehydrogenase and glucose-6-phosphate dehydrogenase in larvae of C. irroratus cultured at the 10-20 C cycle and constant 15 C. Activity of the three enzymes was higher in the first zoeae and the later larval stages than in the intermediate stages for both culture regimes (Fig. 21). However, activity of the three enzyme systems was affected differently by the cyclic temperatures for the larval stages. Lactate dehydrogenase activity was enhanced in the third and fifth zoeal stages and megalops stage at 10-20 C cycle compared to that in larvae at 15 C. The activity of lactate dehydrogenase was significantly increased in the megalops stage compared to that of the earlier zoeal stages, regardless of temperature regime (Table 12). The changes in lactage dehydrogenase activity in the present study probably reflect changes in overall glycolytic activity during larval development. LDH activity in larvae cultured at cyclic regime was substantially higher at the last two stages (Table 12). The malate dehydrogenase activity decreased in all except the fifth zoeal stage at 10-20 C cycle compared to that in larvae at 15 C. The glucose-6-phosphate dehydrogenase activity decreased in the second and third stage zoeae, but increased in the fourth and fifth zoeal and megalops stages at the cyclic regime.

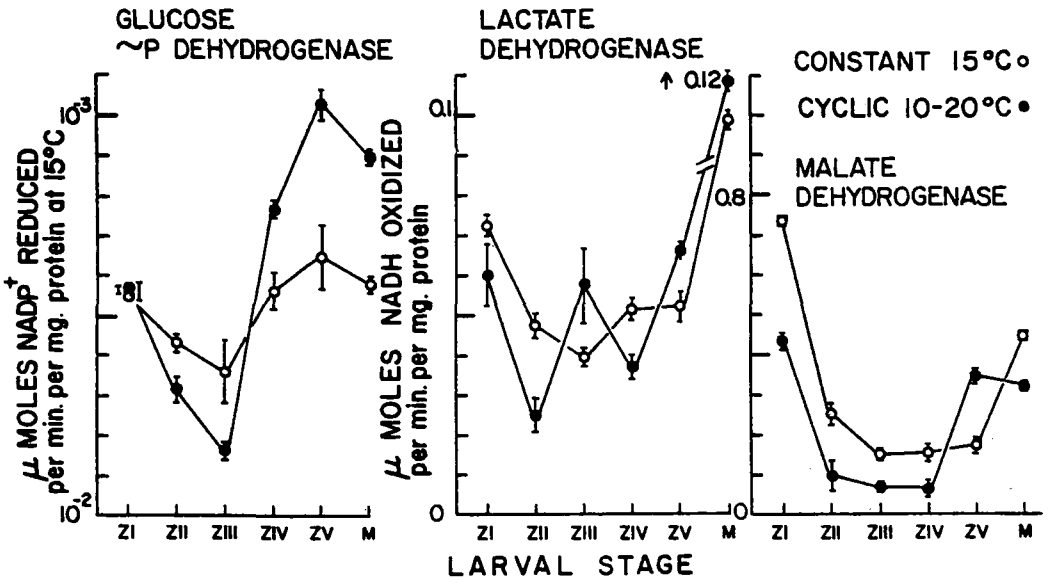


Figure 21. The effects of daily cyclic and constant temperatures on the activities of lactate dehydrogenase, malate dehydrogenase and Glucose-6-Phosphate dehydrogenase in Cancer irroratus larval stages cultured at constant 20 C and 10-20 C daily cyclic temperatures.

TABLE 12. ACTIVITIES OF LACTATE DEHYDROGENASE DETERMINED FROM CRUDE CELL-FREE HOMOGENATES OF CANCER IRRORATUS LARVAE CULTURED UNDER CYCLIC AND CONSTANT TEMPERATURES. ACTIVITY IS EXPRESSED IN μ MOLES NADH OXIDIZED/MIN PER MG LOWRY PROTEIN. ASSAY TEMPERATURE WAS 15C.

| State | Constant 15C | Cyclic 15-20C | Cyclic/constant |
|---|---|---|---|
| Zoea-I | 71.2 | 58.6 | 0.82 |
| Zoea-II | 46.1 | 24.5 | 0.53 |
| Zoea-III | 38.8 | 56.4 | 1.45 |
| Zoea-IV | 50.2 | 35.8 | 0.71 |
| Zoea-V | 51.6 | 103.7 | 2.01 |
| Megalops | 95.1 | 119.7 | 1.26 |

ACUTE TEMPERATURE AND LOW DISSOLVED OXYGEN TOLERANCES

Tolerance limits to acute temperature, alone and in combination with low dissolved oxygen stresses were determined for five zoeal stages and the megalops of Cancer irroratus. Temperature tolerance at saturated dissolved oxygen levels varied with stage, with this variation dependent on exposure time (Fig. 22). Little interstage variation was observed for the 120 minute time interval, with all stages having an $LD_{50}$ for temperature of about 29 C (Table 13). For a 240 minute exposure, there was an overall decline in tolerance and more interstage variation. The second and fourth zoeae were slightly more tolerant (28.2-28.5 C), while the others were less tolerant (27.2-27.5) (Table 13). No significant correlation was found between stage and temperature tolerance to indicate that there was a continuous relationship between morphologically distinct stages and temperature tolerance.

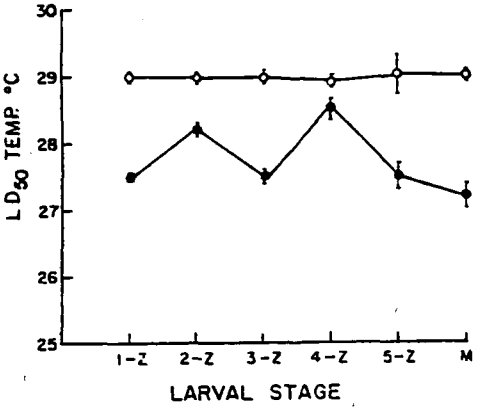


Figure 22. Cancer irroratus. Effect of larval stage on $LD_{50}$ values for temperature. Open circles: 120 min exposure; filled circles: 240 min exposure. 1-5Z; Zoeal stages; M: megalops.

TABLE 13. CANCER IRRORATUS. $LD_{50}$ VALUES FOR TEMPERATURE FOR 120 MIN AND 240 MIN EXPOSURE TIMES, BY STAGE. NO STATISTICALLY SIGNIFICANT CORRELATION WAS FOUND BETWEEN $LD_{50}$ AND STAGE. OXYGEN CONCENTRATIONS WERE KEPT AT SATURATED LEVELS IN ALL TESTS

| Stage | $LD_{50}$ temperature (°C) | |
|---|---|---|
| | 120 min | 240 min |
| 1 | 29.0 ± 0.2 | 27.5 ± 0.1 |
| 2 | 29.0 ± 0.02 | 28.2 ± 0.1 |
| 3 | 29.0 ± 0.1 | 27.5 ± 0.1 |
| 4 | 28.9 ± 0.1 | 28.5 ± 0.2 |
| 5 | 29.0 ± 0.3 | 27.5 ± 0.2 |
| Megalops | 29.0 ± 0.01 | 27.2 ± 0.2 |

Larvae exhibited interstage variation in their pattern of response to low dissolved oxygen tolerance over the experimental temperature range (Fig. 23). The first, second and fourth zoeae showed a different pattern of response, with an increase in tolerance from 10 to 15 C and a decrease from 15 to 30 C (Fig. 23). Tolerance limits were maximum when the test temperature was the same as culture temperature (Fig. 24). This pattern was consistent for both the 120 and 240 min time intervals, with a tendency for lower tolerance limits for the longer time intervals.

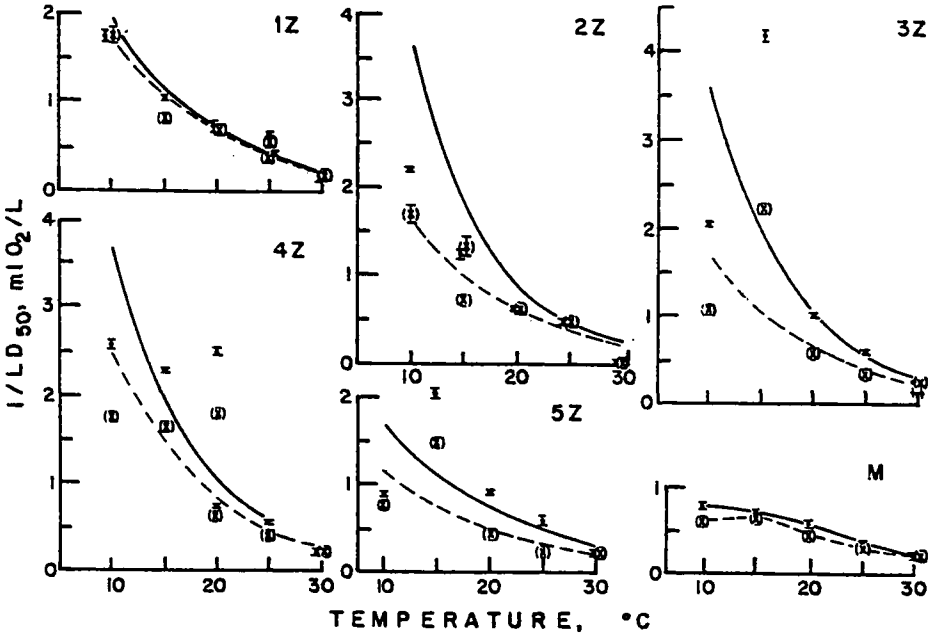


Figure 23. Cancer irroratus. Effect of temperature on $LD_{50}$ value of oxygen for different larval stages. Curves computed from regression equations except for megalops (M); Dots: 120 min exposure (solid line); crosses: 240 min exposure (dashed line). 1-5Z: Zoeal stages.

The megalops stage showed the least temperature-dependent low dissolved oxygen tolerance (Fig. 23). Tolerance decreased from 10-30 C; however, the slope was slight compared to that of other stages. There was no significant difference in tolerance as exposure time was increased from 120 to 240 minutes. Regression analysis did not yield any statistically significant relationship with temperature.

A comparison between larval stages showed the megalops was least tolerant at most test temperatures (Figs. 23 & 24). There was interstage variation in tolerance from 10 to 20 C, however. At the extremes of 25 and 30 C, little difference was observed between stages. At 10 C, the first, second and fourth zoeal stages showed higher tolerance than the other stages. At 15 C, the third stage was most tolerant, while at 20 C, the fourth was the most tolerant. No statistically significant correlation of tolerance with stage was found using either $1/LD_{50}$ = (stage number) + B or log $1/LD_{50}$ = a (stage

number) + b.

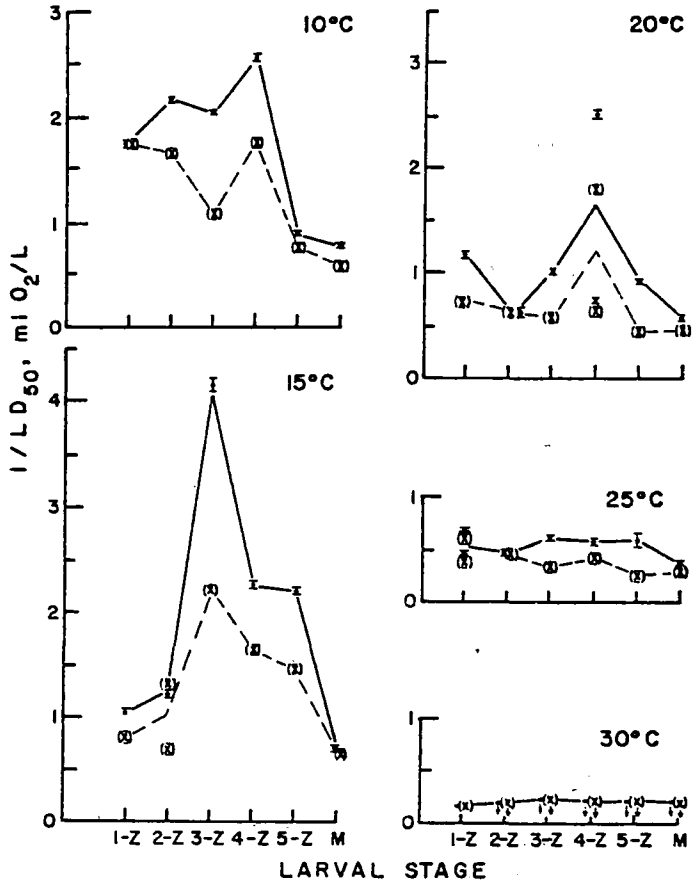


Figure 24. Cancer irroratus. Effect of stage on $LD_{50}$ value for oxygen. Dots: 120 min exposure (solid line); crosses: 240 min exposure (dashed line). 1 - 5Z: Zoeal stages; M: megalops.

## Interspecific Comparison of Temperature Tolerance limits

The larval stages of three species exhibited different thermal tolerance limits to extremes. The upper limits for 50% survival of C. irroratus, H. americanus and P. pugio, each cultured in their respective optimal temperature and salinity combinations, are presented in Fig 25. The temperature tolerance limits for P. pugio larvae were much higher than those for the other stages. Between the two coastal species, C. irroratus larvae exhibited lower temperature tolerance limits than H. americanus larvae. The tolerance limits for successive larval stages remained fairly constant for each species, with the exception of certain stages. The limits for the fourth and seventh stages of P. pugio were 2 and 2.3 C higher, respectively, than for other species. Second stage H. americanus larvae were less tolerant than was any other stage. For C. irroratus larvae, the second and fourth stages

tolerated slightly higher temperatures and the megalops lower temperatures than the other stages. It would appear that although certain stages in the larval development of a species may be slightly more resistant or sensitive to temperature, the general temperature tolerance limits for overall development remains the same for a species and is related to the temperature range of their habitat.

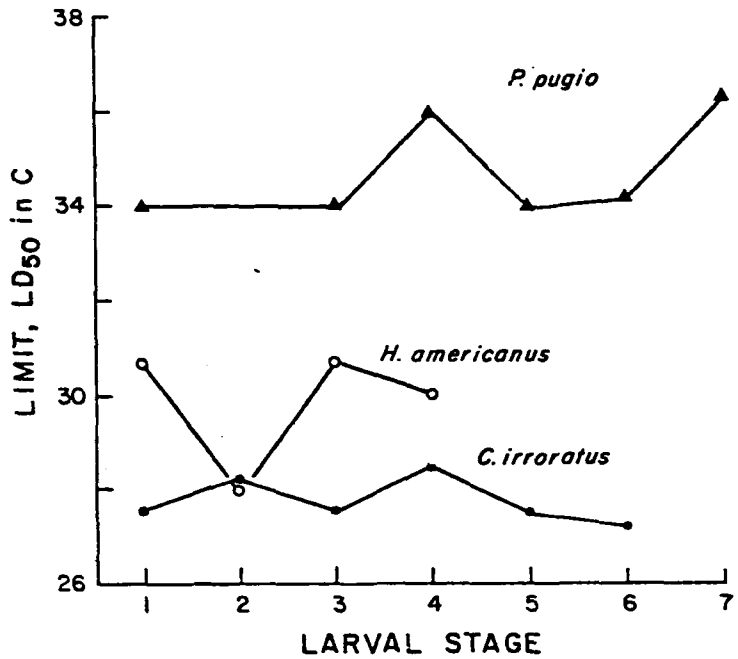


Figure 25. Acute temperature tolerance limits for three species of crustaceans cultured under constant conditions optimal for their survival. C. irroratus, 15°C - 30 o/oo; H. americanus and P. pugio, 20°C - 30 o/oo.

## SECTION 6

## DISCUSSION

Larvae of epibenthic crustacea play an important role in the distribution, gene exchange, pelagic food web and recruitment of young to the adult populations. Generally, larvae are released into the pelagic environment when conditions are optimal for their development and growth. During their pelagic existence larvae are exposed to continuously varying temperatures along with other fluctuating environmental parameters. Larval development occurs within a range of environmental conditions which is characteristic to each species. Interaction of environmental parameters can affect larval development as reflected in varying survival and developmental rates when cultured under different combinations of temperature and salinity (Costlow and Bookhout, 1964; Sastry and Vargo, 1977). A species may exhibit an optimal survival with a given combination of conditions. Then as environmental conditions deviate from this optimum, survival rate is reduced. Limits, as well as the optimal combination for development, vary interspecifically. In the present study, the coastal species (high salinity) completed development over a narrower range of temperature and salinity than the predominantly estuarine species. These limits varied from a single temperature and salinity combination, as seen with C. borealis, to a wide range of suitable temperature and salinity conditions as occurred with P. longicarpus and P. pugio. Limits for complete development may also be a function of the environmental history of the eggs prior to their hatching. Larvae of C. irroratus hatched from eggs incubated during the period they are normally released in nature survived in laboratory culture better than those hatched at other seasons. It appears then that stage of embryonic development at which the eggs are incubated for hatching and their previous thermal history will affect the survival rate, and the limits for complete development.

In addition to these variations, larvae of C. irroratus cultured under constant and daily cyclic regimes also differed in their survival and time required for complete development. Those cultured under a suitable amplitude and rate of temperature change showed an increase in survival compared to those at comparable constant temperatures. Beyond these limits, as with the 15-25 C cylce for C. irroratus, larval development was delayed compared to that at constant 20 C. The affects of daily cyclic and constant temperatures on survival and development time also varied inter-specifically. The estuarine grass shrimp, P. pugio larvae showed no significant differences in either the duration of development or survival at daily cyclic and comparable constant temperatures. Clearly, the affects of fluctuating temperatures from observations on one species should not be generalized for others.

Larvae of geographically separated populations may also exhibit differences in their survival and time required for complete development. For example, larvae of R. harrisii from the New England region developed more slowly than those from southern geographical regions at somewhat similar temperature and salinity combinations. In contrast, the larvae of geographically separated populations of H. americanus and P. pugio showed no pronounced differences in their developmental time at somewhat similar comparable culture conditions (Table 6 and 7). A comparative assessment of latitudinal population differences in the effects of temperature and other environmental factors on larval development and survival would be necessary in utilizing the present results for water quality criteria purposes.

Within the tolerance range of a species, metabolic rate may vary relative to temperature, but each species usually has a characteristic overall response. For example, C. irroratus larvae showed a narrowing of the temperature range for metabolic compensation ($1<Q_{10}<2$) with development, but the temperature range for depression of metabolic rate remained the same. In contrast, C. borealis larvae showed a shift in the temperature range of compensation from colder to warmer temperatures in the later stages. These larvae also had a broader temperature rate for metabolic range compensation than the congeneric species C. irroratus. Larvae of H. americanus were metabolically active over a narrower range and the rate was depressed above 25 C. In contrast, larvae of P. pugio, an estuarine species were metabolically active over a much wider temperature range than those of the sublittoral species and also showed metabolic-rate compensation over wider ranges.

Metabolic response patterns of the larvae also varied when they were cultured under daily cyclic temperatures. A daily cyclic culture regime extended their temperature range for metabolic rate compensation for some of the C. irroratus larval stages compared to those under constant conditions. The cyclic regime also increased larval survival of this species. In contrast, the altered metabolic responses seen in the larvae of H. americanus and P. pugio cultured under daily cyclic regimes were neither consistent nor predictable. Therefore, we see that it is also not possible to generalize the effects of fluctuating thermal regimes on the metabolism, growth and development on larvae of one crustacean species by comparing with the responses by others.

Culture of crustacean larvae under daily cyclic and comparable constant temperatures can also alter biochemical responses as reflected in the qualitative and quantitative changes in free fatty acid methyl esters in larvae of H. americanus. There was a greater tendency for unsaturation of certain chain length (i.e., 16:0) fatty acids in larvae cultured under cyclic regime compared to those cultured under constant temperature. Certain chain length fatty acids present in only trace amounts in larvae cultured at constant temperature were observed in detectable amounts in those cultured under daily cyclic regime.

The activities of enzymes are also affected differently by the thermal culture regime in larvae of C. irroratus. However, the differences in activity of the enzymes in larvae from the two contrasting culture regimes did not follow the same trend, suggesting that enzymes systems in different metabolic sequences are differently affected in each stage by the cyclic temperatures.

(Sastry and Ellington, 1978).

Acute temperature tolerance limits varied intra- and inter-specifically with estuarine species tolerating higher temperatures than the coastal species under saturated oxygen conditions (Sastry and Vargo, 1977). These limits remained fairly constant for all C. irroratus larval stages; some H. americanus and P. pugio stages showed differential sensitivity. However, when low dissolved oxygen and temperature stress were combined more inter-stage variation was evident with C. irroratus.

The metabolism, development and growth and survival of larvae may vary with stage, season of hatching, culture conditions, and geographic origin of the population. These intra-specific variables have to be taken into consideration in the application of laboratory bioassay results for the evaluation of the potential effects of thermal and other pollutants on a natural population or community.

SECTION 7

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Sastry, A. N. Metabolic adaptation of developing larvae of crustacea to the varying thermal environment. XIV Pacific Science Congress, Khabarovsk, USSR, August 20- September 1, 1979.

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

Larvae of six species, Cancer irroratus, C. borealis and Homarus americanus of coastal waters (high salinity), and Palaemonetes pugio, Pagurus longicarpus and Rhithropanopeus harrisii, from the estuarine region (variable salinity) were studied. Larvae were cultured at various combinations of temperature and salinity and highest survival rates and limits for complete development determined. Coastal species have a more restrictive temperature range. Thermal tolerance limits for larvae of the primarily estuarine P. pugio were higher compared to larvae of coastal species, C. irroratus and H. americanus. When temperature and low dissolved oxygen stresses were combined, thermal tolerance limits of C. irroratus larvae were altered. Survival was better for C. irroratus larvae cultured under certain daily cyclic regimes vs. a constant temperature. In contrast, larvae of P. pugio showed no significant differences in either survival or developmental rate when under cyclic vs. constant temperatures.

Metabolic responses of C. irroratus, H. americanus and P. pugio were determined for a series of temperatures. Larvae of coastal C. irroratus and H. americanus were metabolically active over a narrow temperature range. The estuarine species P. pugio and response patterns of larvae cultured at cyclic temperatures differed from those at constant temperatures. Differential effects of daily cyclic vs. constant temperatures also occurred in fatty acid methyl esters in H. americanus and in enzyme systems of C. irroratus larvae.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Crustacea<br>Larvae<br>Physiological Effects<br>Temperature<br>Salinity<br>Oxygen | Larval development<br>Limiting factors<br>Cyclic temperatures<br>Estuarine<br>Coastal | 06F |

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