THERMAL RESISTANCE IN RELATION TO VERTICAL ZONATION IN THREE INTERTIDAL BIVALVE MOLLUSCS

A Thesis Presented To the Faculty of Graduate Studies Dalhousie University

by

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ABSTRACT

Three pelecypod molluscs *Modiolus demissus*, *Mya arenaria*, and *Mytilus edulis* were subjected to acute thermal stress following acclimation variously to 5.0, 15.0 25.0 C and 15, 30‰ S. Mortality responses, for exposures lasting up to 10,000 min, were measured for each of the species-acclimation combinations. A substantial delayed mortality, for most test temperatures, occurred during the first 10-12 days of the post-bioassay recovery period. Times to 50% mortality were calculated at 2-day intervals during the 28-day post-bioassay recovery period.

Thermal resistance lines, for zero-time and the 28th day of post-bioassay recovery, are presented. Zero-day thermal resistance lines were either linear or curvilinear whereas those for the 28th day were invariably linear.

Upper lethal temperatures for a 5760-min (96 hr) exposure are presented for the species, acclimation temperature, acclimation salinity combinations. Analysis of variance indicates that "species" and "acclimation temperature" components are significant whereas the "acclimation salinity" component is not.

The range of upper lethal temperatures for each species in these bioassays is demonstrably higher than the mean ambient field conditions. Thermal resistance capacities

are sufficient to compensate for the diel thermal fluctuations at the collecting sites which sometimes do exceed these experimentally obtained upper lethal temperatures.

INTRODUCTION

A general biological principle states that every species is adapted in some degree to the specific conditions of its environment. By that means, the probable survival of an organism is enhanced across the total scale of physico-chemical conditions which normally occur in its particular range of distribution.

Physiological adaptation is the relationship among the physiological capacities of the organisms and the biotic and abiotic qualities of their environment. Prosser (1964) suggests that the process of physiological adaptation can be examined at two distinct levels. Genetic adaptations are determined and initiated by natural variation and selection during the evolution of a species. Genetic adaptations are properties of populations. The time course of these adaptations is in the order of many generations. Environmentally induced adaptation or acclimatization may be considered as a term embracing those adaptation processes resulting from exposure to local environmental changes (Fisher, 1958). Acclimatization represents the properties of single organisms. The time course of the changes brought about by acclimatization is in the order of minutes, days or weeks. Adaptations due to acclimatization are possible only within a range as prescribed by the genetic compositions of the organism. Genetically induced adaptations among species can be separated from environmentally induced adaptation among species by

acclimation experiments. Genetically induced and environmentally induced adaptations within a species can be separated by breeding experiments in different environments to determine whether or not observed differences in adaptation are persistent.

Physiological adaptations can be studied at three organizational levels. The molecular properties of the biochemical systems of an organism can be investigated (Read, 1963; Brown *et al.*, 1967). The physiological capacities and reactions of certain tissues can be studied (Vernberg *et al.*, 1963; Schlieper, 1966; Dzhamusova, 1967; Zhirmunsky, 1967, 1973). Finally, the physiological reactions of the intact organism can be analysed under various conditions of an environmental variable (Loeb and Wasteneys, 1912; Doudoroff, 1942; Fry *et al.*, 1946; Read, 1967; Read and Cumming, 1967; Kennedy and Mihursky, 1971; Waugh and Garside, 1971; Waugh, 1972).

According to Fry *et al.* (1946), Fry (1947) the limits of an environmental variable within which an animal can survive indefinitely with respect to the effects of that variable is the *zone of tolerance*. The levels defining this zone are the *upper* and *lower incipient lethal* levels for series of acclimations. An animal has a capacity to survive for a finite time the influence of a variable whose level is beyond the levels defined by the zone of tolerance.

Under these circumstances the animal is in the *zone of resistance* with respect to the variable. The animal will be in a state of acute *stress* and its potential for survival will depend upon the intensity and the time interval of exposure to the variable.

Brett (1958) defines stress as:

> a state produced by any environmental or other factor which *extends* the normal adaptive responses of an animal, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced.

Brett (1958) further states that

> by this definition the magnitude of the stress then becomes possible of quantitative expression by an estimate of the chances of survival (where actual losses can be recorded) or by a measure of reduction in capacity for normal performance.

Two major subdivisions have been established within the general context of stress. Brett (1958) defines *discriminate stress* as a stress which applies to individuals, singly within a population and not to the total population; *indiscriminate stress* is a stress which applies to every member of a population and is not discrete in its action.

Physical and chemical stresses are almost invariably indiscriminate stresses. Brett (1958) suggests that indiscriminate stresses may operate in a variety of ways. The extreme effect of any stress is to destroy the animal. In this case the stress is considered to operate as a *lethal stress*. Temperature, pressure and toxicants are examples of potentially lethal stresses. Factors which reduce the ability of the animal to perform normal functions which lead to significantly reduced chances of survival are *inhibiting stresses*. Narcotics, endocrine inactivation and low temperatures fall in this category. Any environmental factor which places an undue burden on an animal, necessitating the rapid or steady release of energy, invokes a *loading stress*. Abnormal osmotic pressure, high temperatures and excessive muscular exertion can induce a loading stress.

Exogenous thermal energy or heat is obviously one of the most important environmental variables that affects the activities of heterothermic organisms through its control of metabolic rate. These organisms are continually subjected to some degree of heat, and it is the magnitude of this degree that is of importance to the organism. A high level of thermal energy can act to induce indiscriminate stress according to Brett's definition. When temperature operates as a lethal stress, the level required to kill an organism depends upon the thermal history of the organism and the physiological status of the organism (Doudoroff, 1942;

Fry, 1947; Orr, 1955; Kinne, 1970) and the intensity and time interval of the stressful thermal exposure (Fry, 1947).

Variations in the ambient salinity in an estuary can be pronounced and with dramatic effects. Butler (1949, 1952) reports mass mortalities of oysters along the Mississippi Coast associated with reductions in ambient salinity. Thomas and White (1969) report mass mortality of estuarine fauna at Bideford, P.E.I. (Canada) associated with abnormally low salinities. In accordance with Brett's (1958) classification of stresses, variations in ambient salinity may operate as an indiscriminate stress when osmotic gradients elicit supranormal metabolic responses.

Modiolus demissus (Dillwyn), *Mya arenaria* (Linné) and *Mytilus edulis* (Linné) have been chosen as typical estuarine intertidal pelecypods of temperate latitudes suitable for an experimental study of physiological reaction to stress resulting from prescribed thermal and osmotic conditions at the organismic level.

The ribbed mussel (*Modiolus demissus*) is classified in the Family Mytilidae. Its distribution ranges from the southern Gulf of St. Lawrence south to Georgia (Bousefield, 1960). At the southern limit of its distribution the ribbed mussel occurs in the lower portion of the intertidal zone (Kuenzler, 1961) whereas at the northern limit it occurs in the higher portion of the intertidal zone (Thomas, 1970).

The normal habitat is in salt marshes where it is commonly attached by byssal threads to buried stems and roots of marsh grass.

The soft-shell clam *(Mya arenaria)* is a member of the Family Myacidae. It occurs from Labrador to North Carolina. At the southern limit of its distribution it is almost exclusively subtidal (Bousefield, 1960). Soft-shell clams near the northern limit of distribution are more commonly intertidal (Thomas, 1970). The customary substrate is muddy sand of intertidal estuaries which are not subjected to strong currents or wave action. Stanley (1970) states that depth of burial for larger clams (≤10 cm) is about 20 cm whereas depth of burial for smaller clams (5 to 6 cm) ranges from about 10 to 20 cm.

The blue mussel *(Mytilus edulis)* is classified in the Family Mytilidae. It ranges from Baffin Island to South Carolina (Bousefield, 1960). It is almost exclusively subtidal at the southern limit of its range but occurs intertidally to several meters subtidally in the northern portion of its distribution range where rocky surfaces are the preferred substratum, but shells or stones may serve as nuclei for the formation of large clumps of individuals on intertidal mud flats.

The experimental phase of this study consisted of the determination of upper lethal temperatures for designated sets of acclimation conditions in the three species according to the general procedures developed by Fry and coworkers (1946, 1947). Certain modifications to such procedures had to be introduced because recognition of the point of death in these cryptic animals is necessarily achieved only indirectly. Subsequently, some attention was diverted from the assessment of lethal temperatures *per se* to an evaluation of latent mortality which attends some of the animals several days after a potentially lethal stress had been removed.

METHODS AND MATERIALS

Ecological conditions of Petpeswick Inlet

Physical description of Petpeswick Inlet

Petpeswick Inlet (lat 44° 45′ and long 63° 10′) is situated 35 km east of Halifax, Nova Scotia (Fig. 1). Loucks and Sadler (1971), Platt and Irwin (1972) describe it as a small, shallow, marine inlet 9.5 km long with a surface area of 7.75 sq km and a maximum depth of 24 m. It is open to the sea by a long narrow channel with a minimum depth of 2 m at low tide and a width of 50 m at its narrowest point. Tides are of the semi-diurnal type, 0.6 to 2.0 m in amplitude. At low tide approximately 70% of the surface area of the inlet is exposed. Extensive mud flats and eel grass *(Zostera marina)* beds predominate in the intertidal area.

Summer thermal conditions of Petpeswick Inlet

Air and water temperatures in Petpeswick Inlet were obtained from various sources. Mean monthly air temperatures for Petpeswick Inlet (Fig. 2a) were estimated from the average of the mean monthly temperature at Shearwater, Nova Scotia (35 km SW of Petpeswick Inlet) and at Ecum Secum, Nova Scotia (130 km SE of Petpeswick Inlet). The mean monthly temperatures for Shearwater and Ecum Secum were monthly means for the period from 1931 to 1960 (Anonymous, 1967).

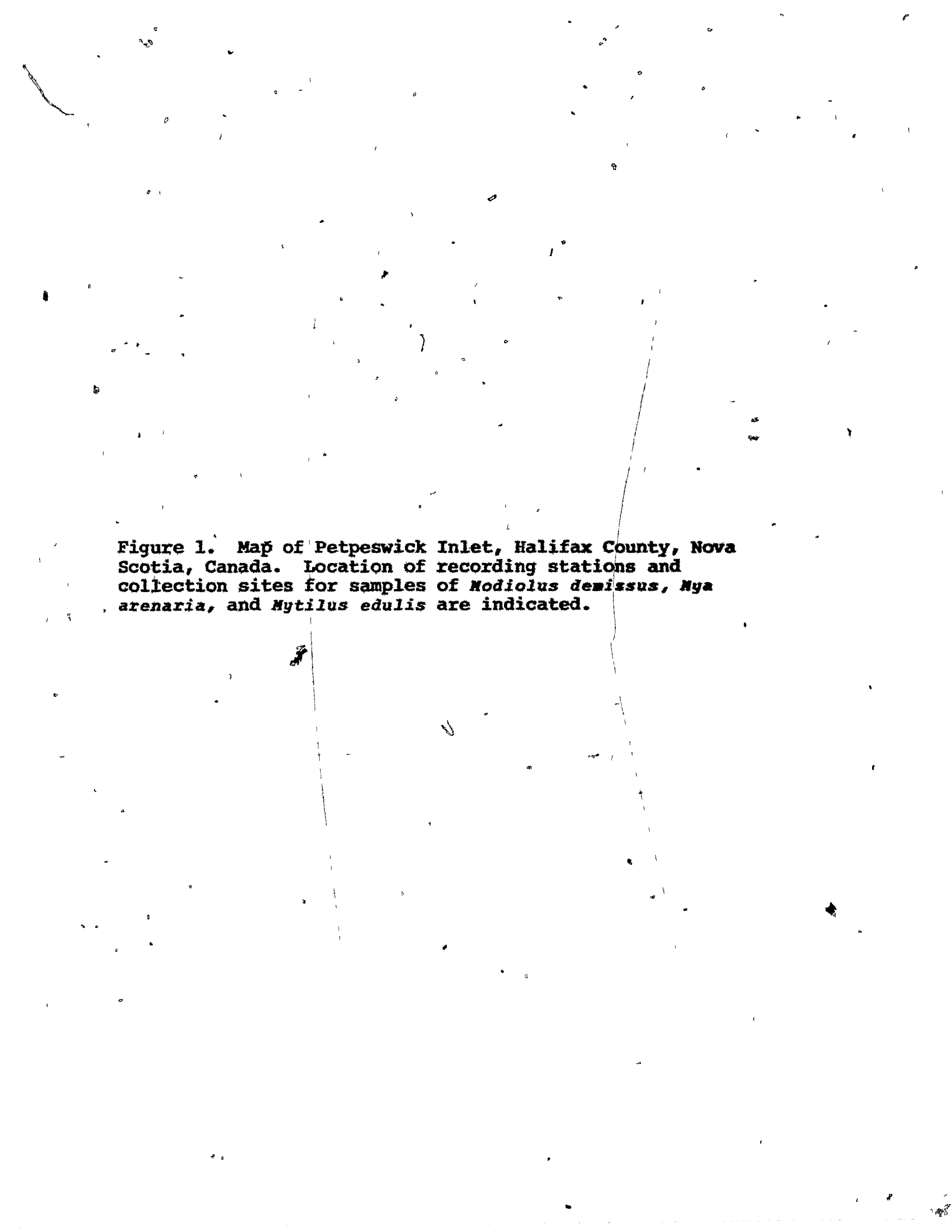


Figure 1. Map of Petpeswick Inlet, Halifax County, Nova Scotia, Canada. Location of recording stations and collection sites for samples of *Modiolus demissus*, *Mya arenaria*, and *Mytilus edulis* are indicated.

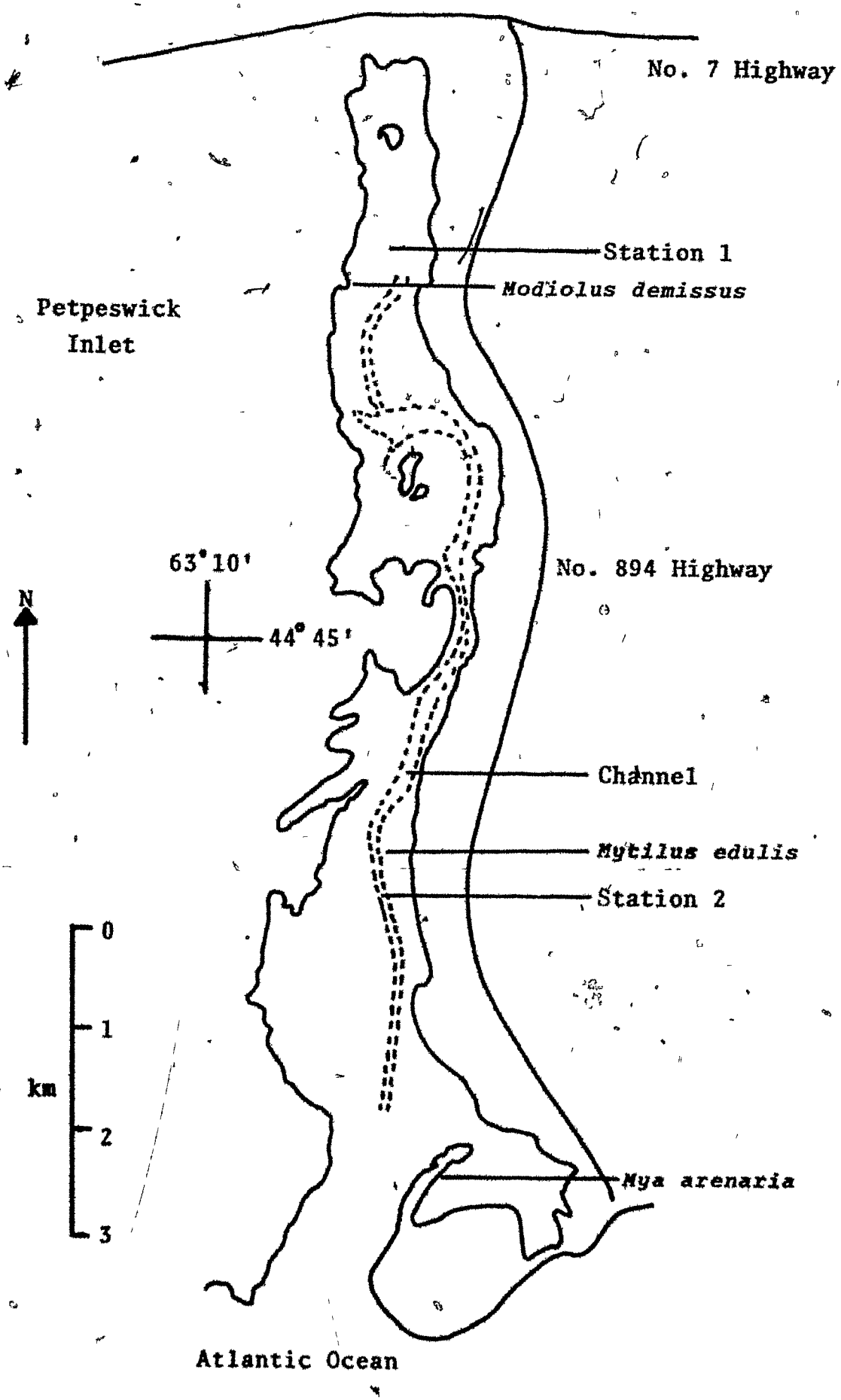
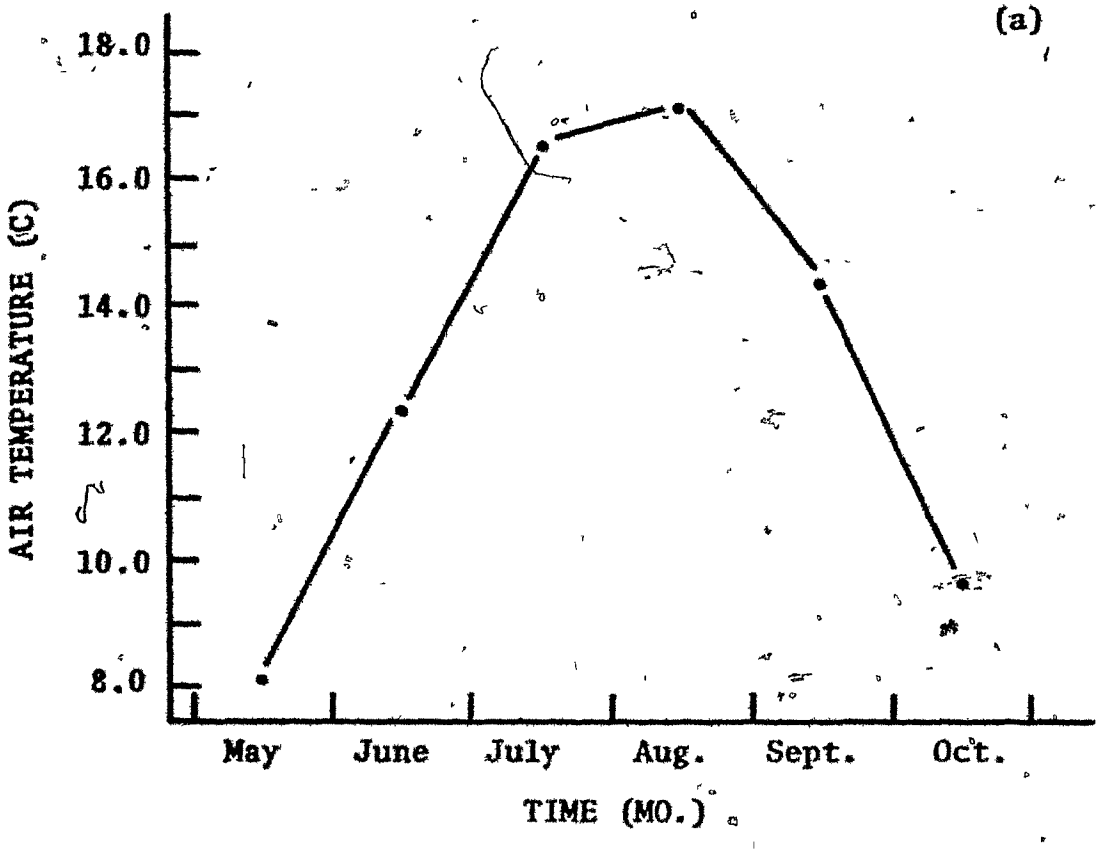
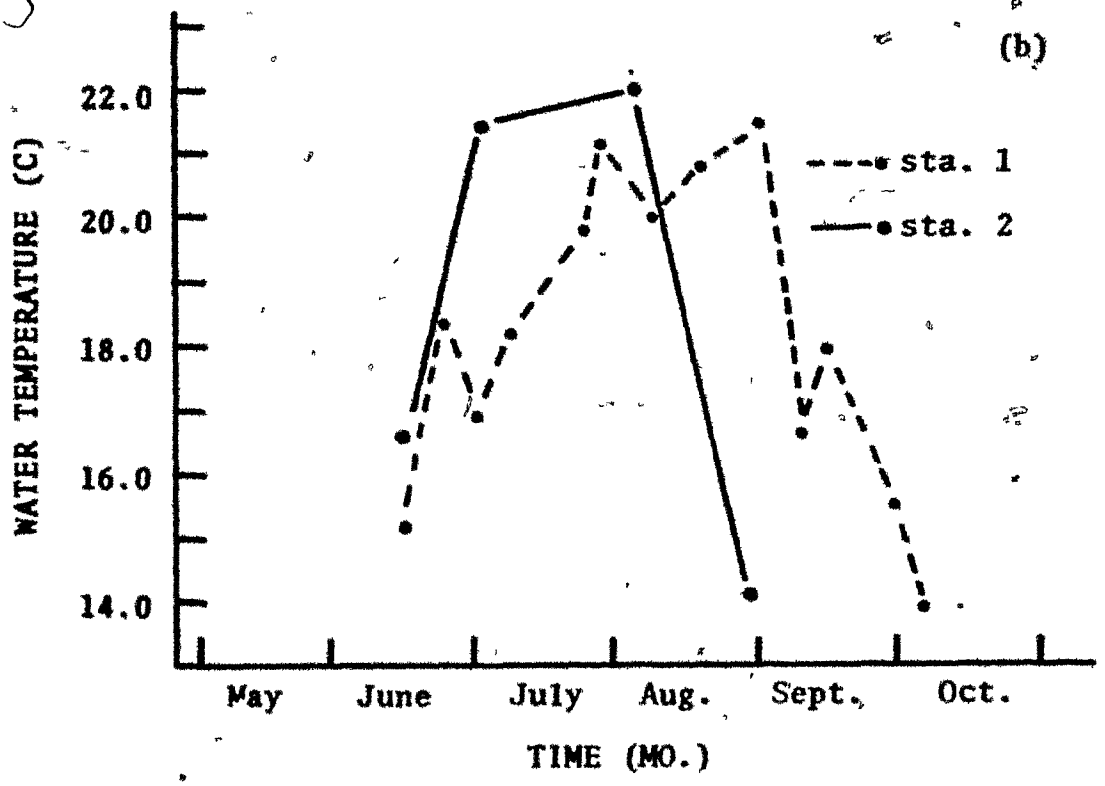

No. 7 Highway
Station 1
Modiolus demissus
Petpeswick
Inlet
63°10'
No. 894 Highway
N
44° 45'
Channel
Mytilus edulis
Station 2
0
1
km
2
3
Mya arenaria
Atlantic Ocean

Figure 2(a). Estimates of monthly mean temperatures for Petpeswick Inlet, Nova Scotia (Canada). The estimates were obtained by averaging the monthly mean temperatures for Shearwater, Nova Scotia and Ecum Secum, Nova Scotia for the period from 1931 to 1960.

Figure 2(b). Surface water temperatures at station 1 and station 2 in Petpeswick Inlet during the period from May 1971 to October 1971.


(a)
AIR TEMPERATURE (C)
18.0
16.0
14.0
12.0
10.0
8.0
May
June
July
Aug.
Sept.
Oct.
TIME (MO.)


(b)
WATER TEMPERATURE (C)
22.0
20.0
18.0
16.0
14.0
sta. 1
sta. 2
May
June
July
Aug.
Sept.
Oct.
TIME (MO.)

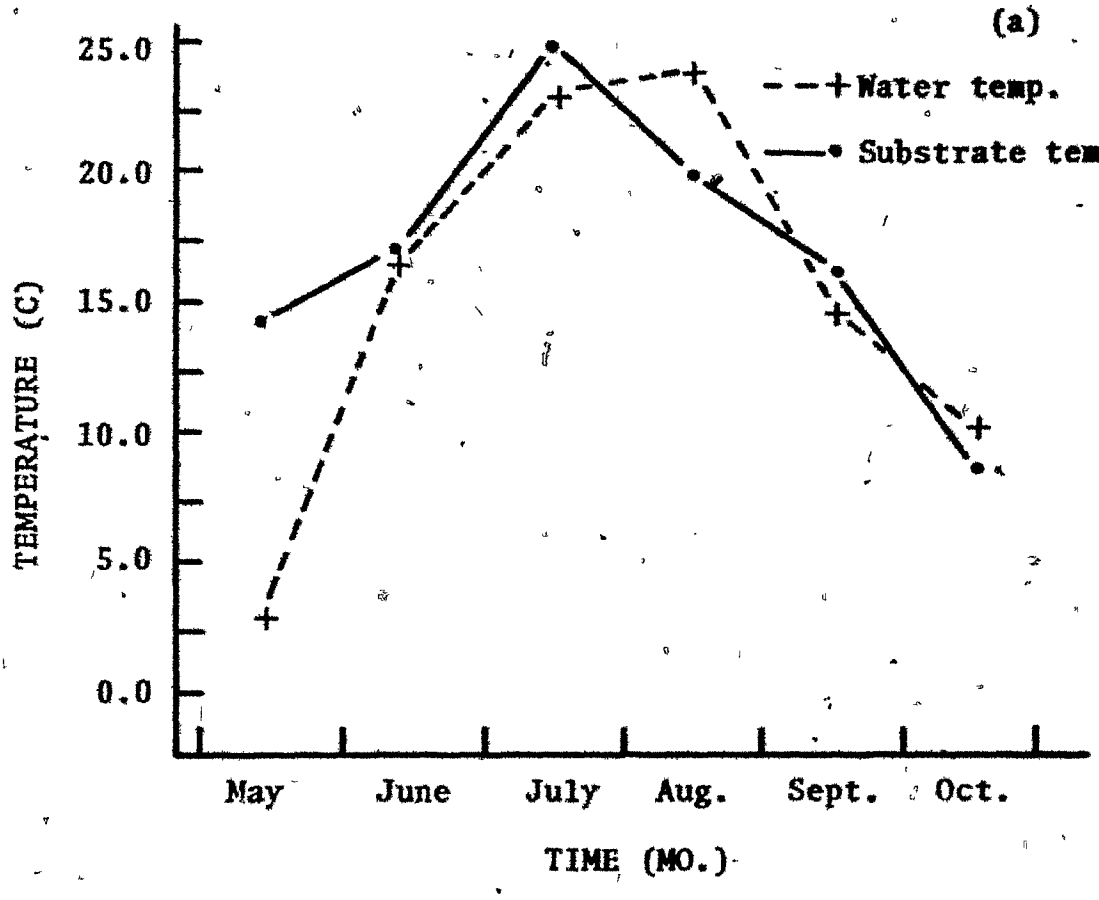
Surface water temperatures for station 1 were obtained from Platt and Irwin (1972) (Fig. 2b). Surface water temperatures for station 2 were obtained from Loucks (1973) (Fig. 2b).

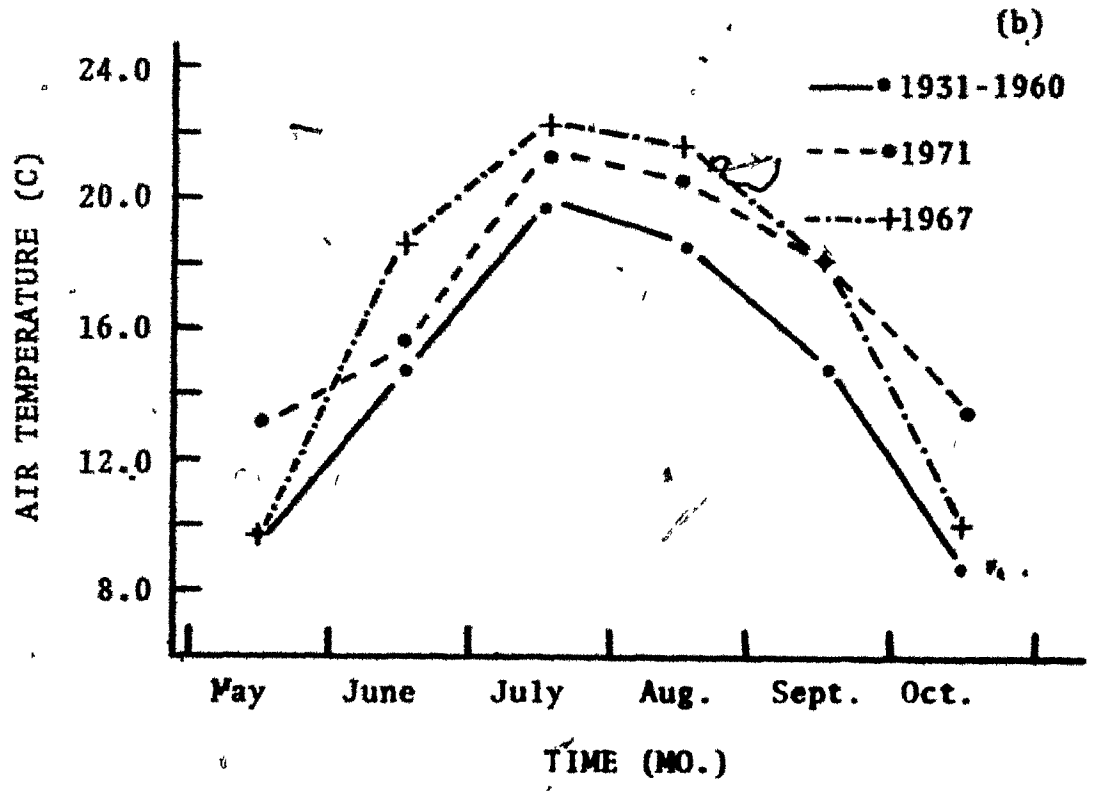
Information is not available on substrate temperatures in Petpeswick Inlet, However, Waugh (1972) obtained substrate temperatues for a population of ribbed mussels which were located in the intertidal zone of Bideford River, a similar estuarine habitat, in Malpeque Bay, Prince Edward Island. These observations were made during the summer of 1967 (Fig. 3a). Thomas (1970) reported the mean monthly water temperatures for a location adjacent to this ribbed mussel population (Fig. 3a). Mean monthly air temperatures in the same geographical location were obtained for the summers of 1967 and 1971 (Fig. 3b). This information was provided by the Atmospheric Environment Service, Environment Canada. Figure 3b presents the monthly mean temperatures of the mean monthly temperatures (1931 to 1960) for Summerside, Prince Edward Island, Canada (40 km SE of Ellerslie, Prince Edward Island).

Figure 4 shows the substrate temperatures for a population of ribbed mussels located in Bideford River, Prince Edward Island. The time interval covered by this information was from July 18, 1967 to July 24, 1967.

Figure 3(a). Mean monthly water and substrate temperatures (1967) for a population of *Modiolus demissus* located in Bideford River, Prince Edward Island (Canada).

Figure 3(b). Mean monthly air temperatures (1967, 1971) for Ellerslie, Prince Edward Island (Canada) and monthly mean air temperatures of the mean monthly air temperatures for the interval from 1931 to 1960, for Summerside, Prince Edward Island (Canada).


(a)
25.0
20.0
15.0
10.0
5.0
0.0
TEMPERATURE (C)
Water temp.
Substrate temp.
May
June
July
Aug.
Sept.
Oct.
TIME (MO.)


(b)
24.0
20.0
16.0
12.0
8.0
AIR TEMPERATURE (C)
1931-1960
1971
1967
May
June
July
Aug.
Sept.
Oct.
TIME (MO.)

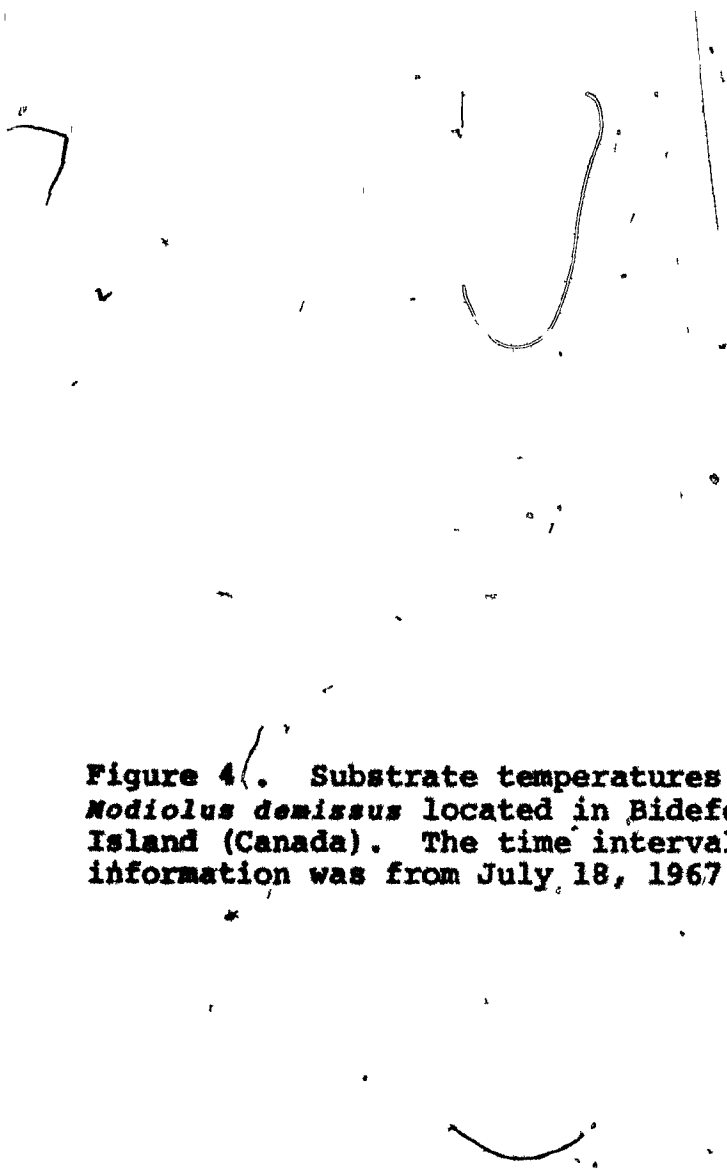
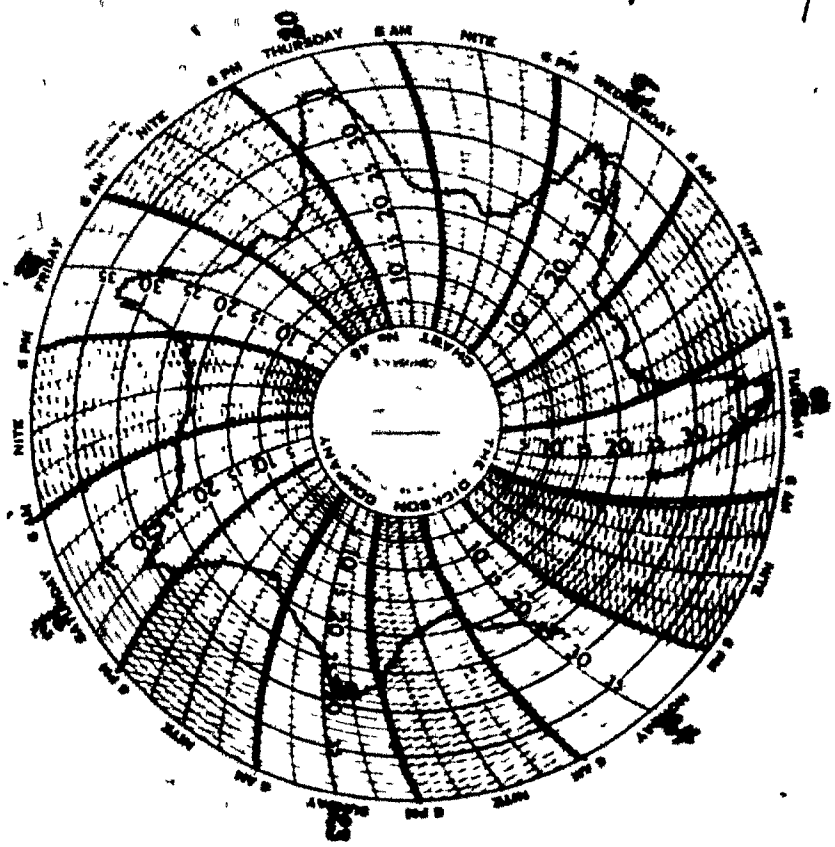


Figure 4. Substrate temperatures for a population of *Modiolus demissus* located in Bideford River, Prince Edward Island (Canada). The time interval covered by this information was from July 18, 1967 to July 24, 1967.


THURSDAY
8 AM
NITE
8 PM
FRIDAY
SATURDAY
SUNDAY
MONDAY
TUESDAY
WEDNESDAY
THE DICKSON COMPANY

Description of collection sites

Clams and mussels used for all experiments were collected from Petpeswick Inlet. Locations of the collecting sites are indicated in Figure 1.

The ribbed mussel population was located in a zone of dense growth of the intertidal grass *Spartina alterniflora*. This vegetation provides the mussels with a considerable degree of insulation from solar heat during the summer. The zone of the ribbed mussel population had a horizontal width ranging up to 10 m but mostly less than 3 m. The lower edge of the population was approximately 0.1 m vertically below mean low water with the upper edge 0.4 m vertically above mean low water (Figure 5).

Estimates of population density were obtained by counting the number of animals within a 0.33 x 0.33 m frame (area 0.1 m^2). Density of animals ranged from 0 to 45. The average, for 45 samples, was 23 per 0.1 m^2.

The soft-shell clam population was located in a substrate of coarse sand and fine gravel. This population had a horizontal width of approximately 4 m beginning at mean low water line and through a vertical drop of 0.2 m (Figure 5).

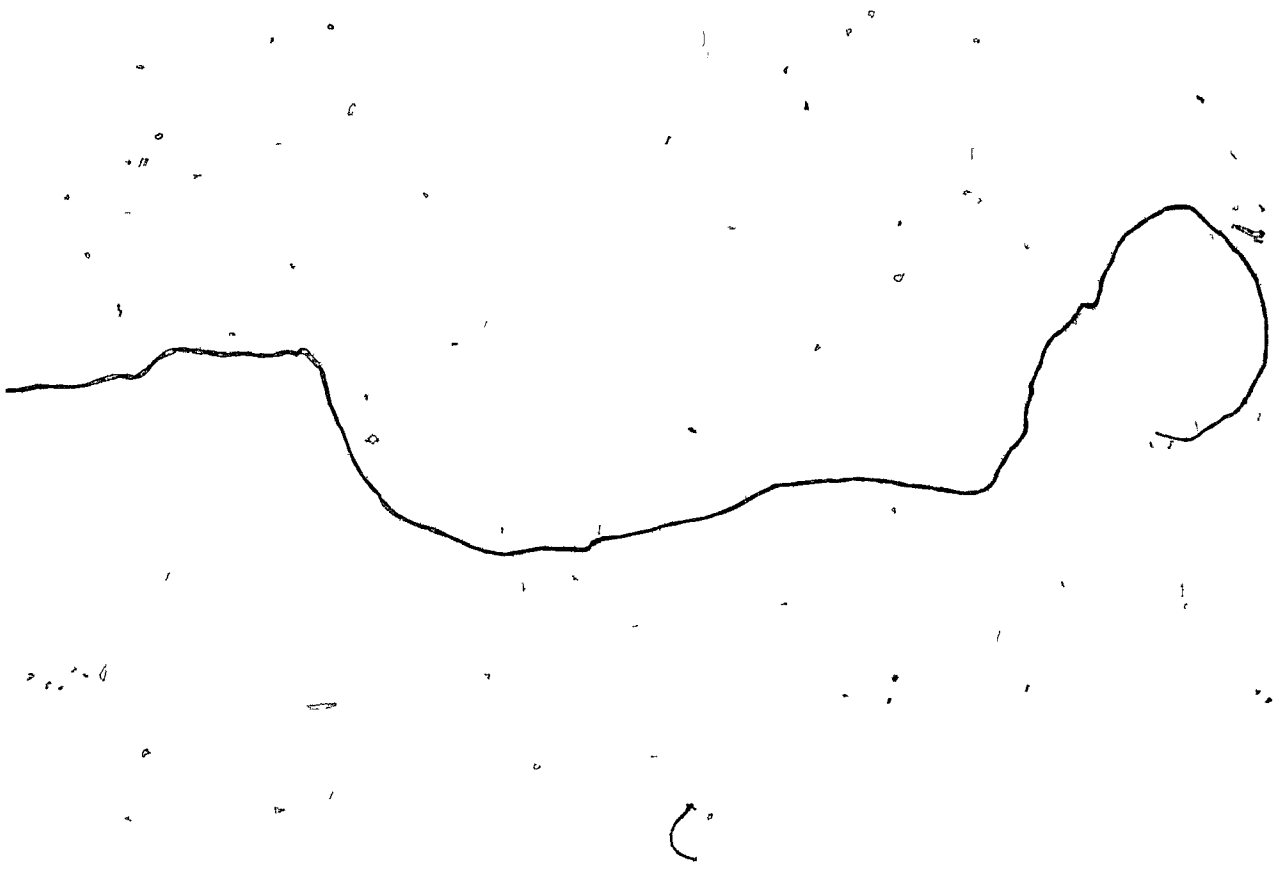
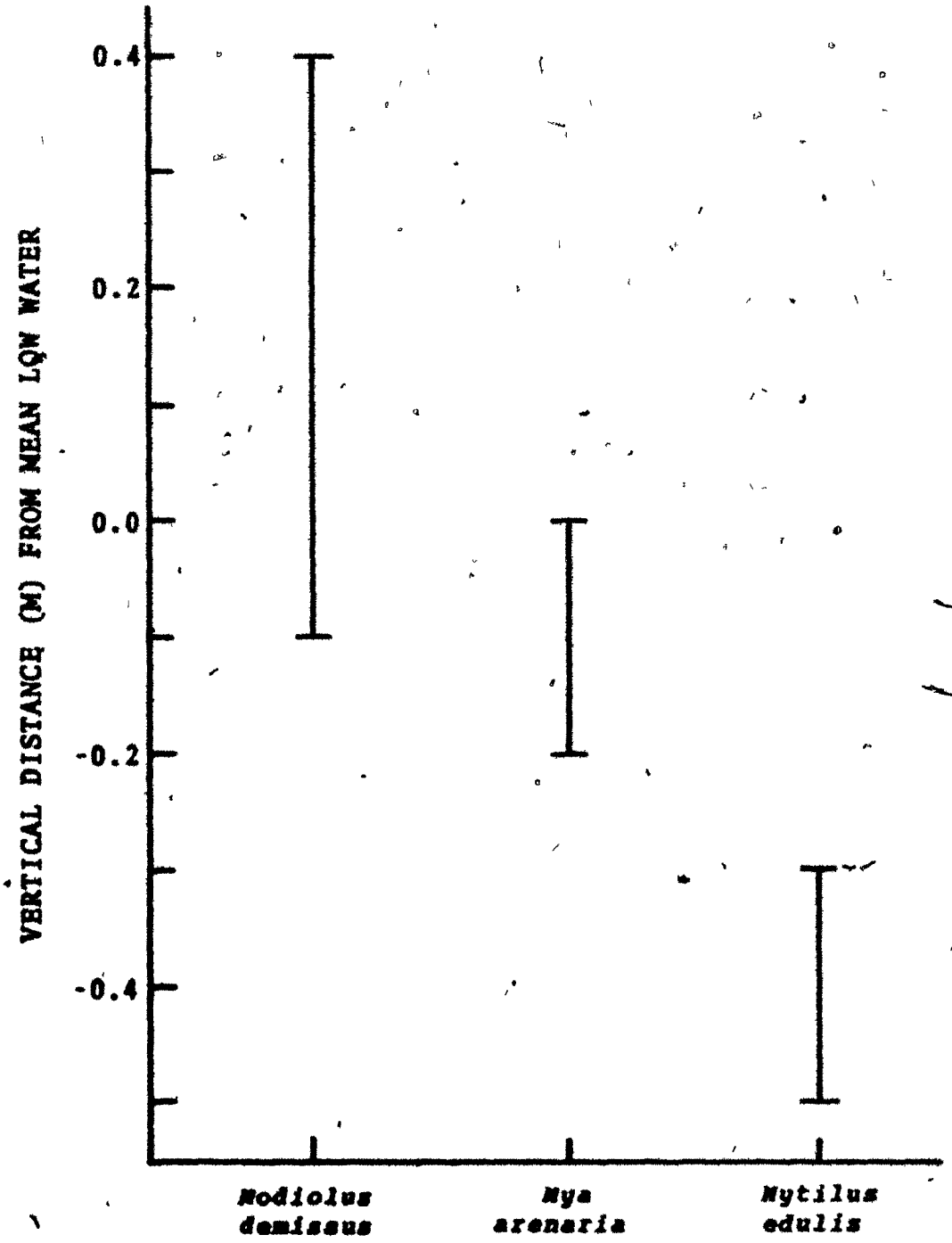


Figure 5 . Diagram indicating the vertical location of *Modiolus demissus*, *Mya arenaria*, and *Mytilus edulis* in relation to mean low tide. Approximate intertidal range of each species is indicated.


VERTICAL DISTANCE (M) FROM MEAN LOW WATER
0.4
0.2
0.0
-0.2
-0.4
Modiolus demissus
Mya arenaria
Mytilus edulis

Population density estimates were obtained in the manner described for the ribbed mussel. Counts ranged from 0 to 36 per 0.1 m^2 with an average, taken over 20 samples, of 16 per 0.1 m^2.

The blue mussel population was located in an extensive mud flat which was covered with eel grass (*Zostrea marina*). Substrate consisted of extremely fine sand and decaying vegetation. The population exhibited a horizontal distribution which frequently exceeded 50 m, however, the highest population densities occurred in narrow bands (5m) along the edges of the water channels in the mud flats. The lower edge of the most densely populated zone was 0.5 m below mean low water. The upper edge was 0.3 m vertically below mean low water (Figure 5).

Population densities were difficult to determine due to the extreme degree of contagious distribution exhibited by blue mussels. However, densities in the highly populated zones ranged from 0 to 46 per 0.1 m^2 with the average for fifty samples, being 22 per 0.1 m^2.

Care and feeding of collected animals

Samples were removed from the collection site as required, with care being taken not to damage them. They were then transported directly to the Life Sciences Centre, Dalhousie University, where they were placed in aquaria. These latter facilities consisted of fiberglass tanks

measuring 1.5 m by 1.5 m by 0.9 m deep. Each tank was supplied with continuously flowing filtered sea water and compressed air. Sea water temperature at all times was slightly above ambient at source, the Northwest Arm, Halifax Harbour. Approximately 1,000 animals were placed in each tank. Daily food rations consisted of 500 ml of *Phaeodactylum* which had a density in excess of 6 million cells per ml. Photoperiod was held at 14 hours light and 10 hours darkness.

Acclimation

Acclimations were performed in a laboratory in the Biology Department, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia. Continuously flowing sea water was mixed with dechlorinated fresh water, via a dilution apparatus, in the appropriate proportions to achieve the desired salinities. This water was subsequently fed by gravity from a head tank to the acclimation tanks. Each acclimation tank was of fiberglass construction and measured 0.9 m by 0.6 m and 0.5 m deep. The flow rate in each tank was maintained at approximately 500 ml per minute. Temperature regulation was achieved by the use of "Jumo" contact thermometers in conjunction with triac semi-conductor relays which controlled either a heating element or a refrigerating compressor. The choice of unit depended upon the temperature of the incoming water and upon the desired level of thermal acclimation.

Animals for acclimation were taken from the holding facilities and placed in other tanks in which the temperature and salinity were adjusted to correspond with those of the holding conditions. Approximately 500 animals were placed in each acclimation tank. Temperature and salinity of the acclimation tanks were then adjusted by 0.5 degrees and 1‰ S per day until the desired levels of temperature and salinity were achieved. The animals were then held at these levels for an additional two weeks before being subjected to bioassay. Each acclimation tank received a total of 500 ml of *Phaeodactylum* culture per day which had a density in excess of six million cells per ml. The photoperiod was controlled manually and approximated 14 hrs light and 10 hrs darkness.

Acclimations were performed on samples of ribbed mussels, soft-shell clams and blue mussels, at three temperatures (5 C, 15 C and 30 C), and at two salinities (15‰ and 30‰). This resulted in a total of 18 acclimation series.

Apparatus for Bioassay

Bioassays were performed in glass aquaria, 0.44 m x 0.22 m x 0.28 m deep. Standpipe levels were adjusted so that each aquarium contained 22 l. Water from the head tank flowed into each unit at the rate of 80 ml per min. The time interval for partial replacement of water in each test aquarium was determined from information supplied by Sprague (1972). Maintenance of the stated flow rate would result in 99% replacement every 24 hours.

The thermostat apparatus consisted of a triac semi-conductor relay connected to a "Jumo" contact thermometer and a 300 w calrod heater coated with epoxy resin paint (Fig. 6). The base of each test aquarium was placed on a board of the same area which was marked into 50 equal squares. Each square was assigned a number and then a rank so that the grid ultimately consisted of 50 randomly identified squares. Those squares that were ranked from 1 to 10 were painted red. This procedure was repeated for those squares that were ranked from 11 to 20 except that the squares were painted white. This procedure was repeated using different colours until all squares were coloured in five series each containing 10 randomly positioned squares.

Bioassays

The first step in the bioassay procedure consisted of unselectively drawing approximately 100 animals from an acclimation tank. A sample of 50 animals, all of which were in excess of 30 mm in length, were selected from this group. The selected sample of animals was then placed in a test aquarium. The test aquarium contained water which had been heated to a predetermined temperature. Salinity of the test aquarium was regulated to correspond to the salinity of the acclimation tank from which the sample was drawn. Each animal was placed over a randomly positioned square. Subsamples of 10 animals each were removed from a colour unit at arbitrarily determined time intervals. The procedure for bioassays was repeated until several subsamples exhibited

Figure 6. Bioassay equipment

(a) solid state relay
(b) salt water line
(c) contact thermometer
(d) calrod heater
(e) air stone


(a)
(b)
(c)
(d)
(e)

initial mortality responses that ranged from 0% to 100%. In all bioassays, test temperature, subsample exposure period, and subsample mortality were recorded. Also, size measured as length, width and thickness of ribbed mussels and blue mussels and length and width of soft-shell clams were recorded for each subsample.

This procedure was repeated for a range of fixed temperatures for each acclimation series. The ultimate aim was to establish for each series, a range of test temperatures: the highest which would provide a range of mortality responses for approximately 100 to 500 minute exposures, and the lowest which would give a range of mortality responses for exposure extending from 8,000 to 10,000 minutes, and temperatures which would yield intermediate values.

Post-Bioassay Recovery Period

Mortality determinations were conducted on individuals of each subsample as they were removed from the test tank. An animal was considered to be dead if the mantle failed to contract when stimulated with a glass rod. Those animals which appeared to be alive were then placed in a perforated 250 ml plastic beaker suspended in the acclimation tank from which these animals had been drawn. In essence, a group of animals was acclimated to a specific set of temperature and salinity conditions, removed and subjected to bioassay,

and then returned to their original acclimation conditions for further observation of possible subsequent mortality in each subsample.

Routine mortality determination was then made on the remaining animals in each subsample at two day intervals for the next 28 days. Animals dead at any inspection were removed and measured. In addition, the time interval in which they died during the post-bioassay recovery period was recorded.

Control experiments were used to determine natural mortality during the bioassay and post-bioassay recovery periods. The procedure was to place a 250 ml perforated beaker, containing 10 acclimated animals, ir the appropriate acclimation tank. Five replicates were used. Mortality in each container was checked at two-day intervals during the bioassay and post-bioassay recovery periods.

Statistical procedures

The data from a bioassay consists of mortality responses, expressed as percentages, for a series of time exposures at a fixed temperature level. The statistical procedure appropriate for handling dose - response data is probit analysis (Finney, 1971). In this analysis, the independent variable (time) is transformed into logarithms and the dependent variable (percent mortality) is transformed

into probits. These transformations normalize the data. The analysis is then conducted to reduce the data to the form

$$y = a + b(x - \bar{x})$$

which Fry (1947) describes as a time-mortality curve.

The next step consists of determining the time to 50% mortality for each test temperature in the series. This is done by solving this equation for $y = 5.0$ (probit 5.0 = 50%) for a series of bioassays. The result is a value which expresses the duration of exposure, at a specific test temperature, which is required to bring about 50% mortality in a sample of animals.

Regression equations were determined for times to 50% mortality and test temperatures for each acclimation series. In this case, log time is represented on the x-axis and test temperature is represented on the y-axis. The logarithmic transformation of time is employed to normalize the data so that linear regression analysis can be performed. Generally, this reduces the data to a first order polynomial equation in the form of

$$y = a + b(x - \bar{x})$$

which has been described by Fry (1947) as a resistance line.

Higher order polynomial equations were used to describe the thermal resistance lines if they provided an improved fit by reducing the mean square due to deviations

about the regression. These polynomial equations describe the relationship between the test temperature and the time to 50% mortality over a range of test temperatures for a specific set of acclimation conditions.

Since for each subsample, percentage mortality was determined and accumulated at two-day intervals during the post-bioassay recovery period, time to 50% mortality was determined for each of these inspection points. Thermal resistance lines were then established for zero-time post-bioassay recovery and for the 28th day of post-bioassay recovery in each of the three species for the six various combinations of thermal and osmotic acclimations.

Thermal resistance lines were also determined for times to 10% mortality and test temperatures for the acclimation series in each species which showed the highest upper lethal temperature for the 28th day of post-bioassay recovery.

There are two parameters which can be used to describe a thermal resistance line. These are regression coefficient and unadjusted mean. An upper lethal temperature for a 50% mortality response can be obtained from the equation of the thermal resistance line by solving the equation for a specific time exposure. This is equivalent to adjusting the unadjusted mean of x to a specific level of x for the thermal resistance line equation. The thermal

resistance line can now be characterized by a regression coefficient, and an adjusted mean which is in effect an upper lethal temperature for a specified time interval of thermal exposure.

Statistical comparisons among thermal resistance lines are based on adjusted means. Therefore, upper lethal temperatures had to be calculated for a specific time interval of thermal exposure. The initial experimental design called for upper lethal temperatures for 10,000 min thermal exposures. The subsequent occurrence of post-bioassay mortality necessitated the calculation of upper lethal temperatures for a thermal exposure of lesser duration. The longest thermal exposure for which data were available from all thermal resistance lines was 5760 min. Therefore, upper lethal temperatures for a 50% mortality response are based on a thermal exposure period of 5760 min.

Before statistical tests can be conducted on the differences among resistance lines, it is necessary to test for homogeneity of variances. Bartlett's test (Li, 1964) was used to test the null hypothesis that the variances of k populations are equal. If this hypothesis is accepted, further analyses may be conducted using parametric statistical analyses.

Statistical analyses of the parameters of the thermal resistance lines resulting from the 18 acclimation combinations

were conducted in accord with the following scheme. Initially, factorial analysis of variance was performed on the upper lethal temperatures to determine the separate effects of the treatments. The test for homogeneity of adjusted means was performed to test the hypothesis that the upper lethal temperatures are equal. If the hypothesis is rejected, then the Student-Newman-Kuels Test (Sokal and Rohlf, 1969) for multiple comparisons among upper lethal temperatures must be used. The results of this test indicate whether specific comparisons made between any two lethal temperatures are significantly different.

Regression coefficients were examined by factorial analysis of variance to determine the separate effects of the treatments. Since there were no replicates, "within-cell" variances could not be determined. Therefore, the significance levels of the treatment interactions are not statistically reliable.

Regression coefficients were further examined for homogeneity with the procedure described by Li (1964). In this case the null hypothesis being tested is that all regression coefficients are equal. Rejection of this hypothesis requires further analysis of the data by the Simultaneous Test Procedure for differences among a set of regression coefficients (Sokal and Rohlf, 1969).

Upper lethal temperatures and regression coefficients for thermal resistance lines representing 10% and 50% mortality, for the prescribed acclimation conditions, were statistically compared by using Students t-test.

RESULTS

Results of the experiments on control animals revealed a cumulative mortality of 10% or less during the combined bioassay and post-bioassay recovery periods. It is assumed that such a mortality response level is not a result of thermal stress. Therefore, Abbott's formula (Finney, 1971) was not used to adjust experimental mortality responses at higher temperatures.

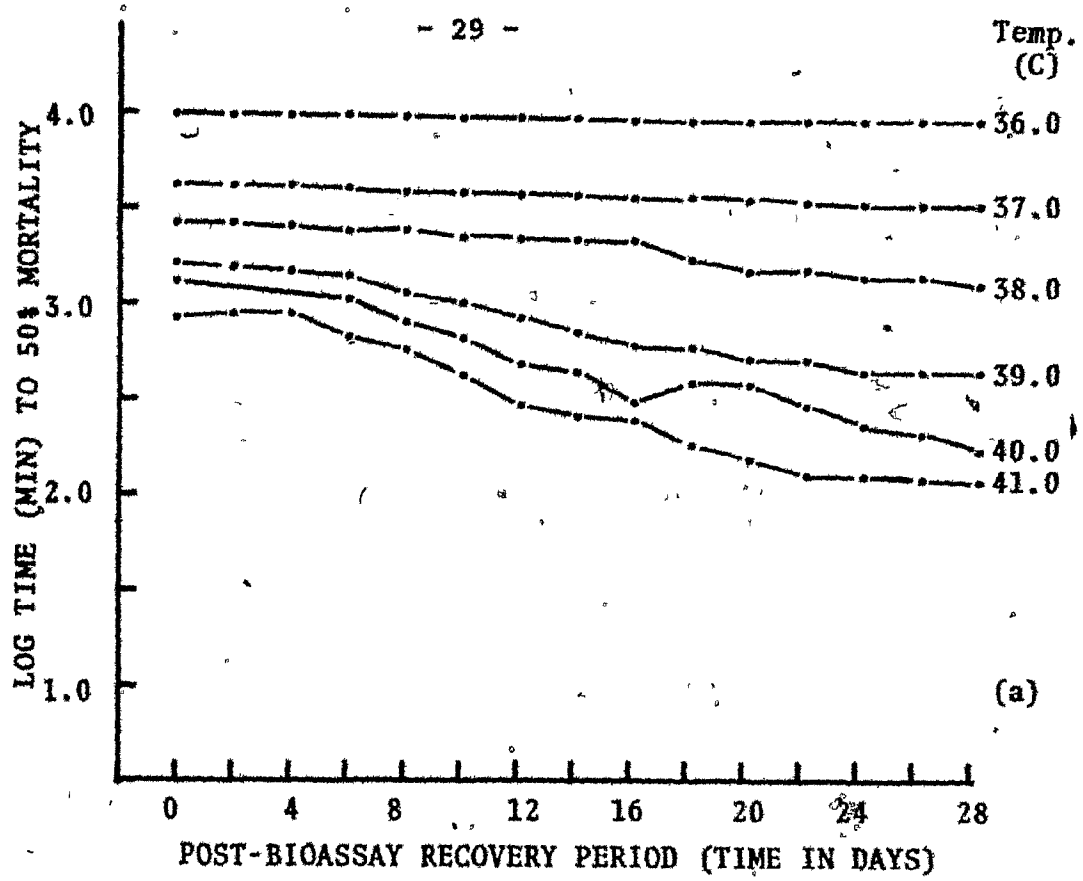
Figures 7 to 24 (part a) show the times to 50% mortality, during the 28 day post-bioassay recovery period, for different test temperatures in the 18 acclimation series.

Logarithmic transformation of the times to 50% mortality resulted in normalization of the data for the 28th day of post-bioassay recovery. Linear regression analysis was then conducted on the data from each set of acclimation conditions. The resulting thermal resistance lines are shown in Figures 7 to 24 (part b).

Similar transformations for the times to 50% mortality for zero-time post-bioassay recovery period did not result in normalization of the data for all sets of acclimation conditions. First and second order polynomial equations were then applied to the data from each set of acclimation conditions in order to provide a line which adequately described the relationship between time to 50% mortality and test temperature over a range of test temperatures. Regression

Figure 7(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Modiolus demissus* acclimated to 15‰ salinity, 5 C and tested at 15‰ salinity.

Figure 7(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Modiolus demissus* acclimated at 15‰ salinity, 5 C and tested at 15‰ salinity.


Temp. (C)
36.0
37.0
38.0
39.0
40.0
41.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
(a)
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)

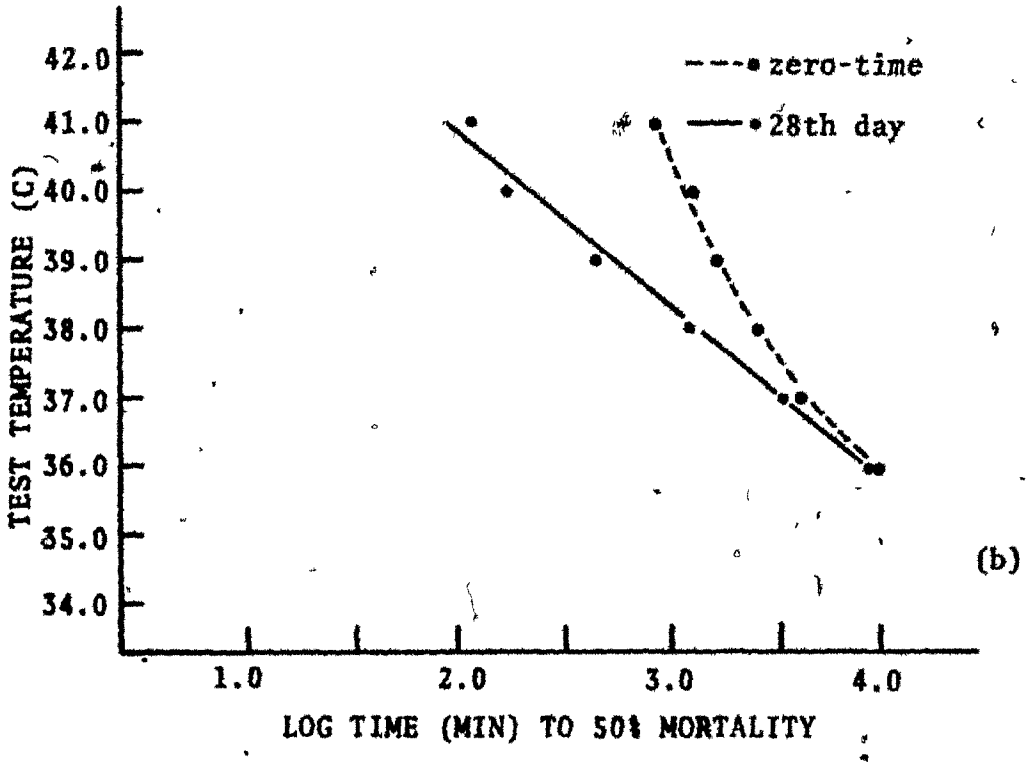
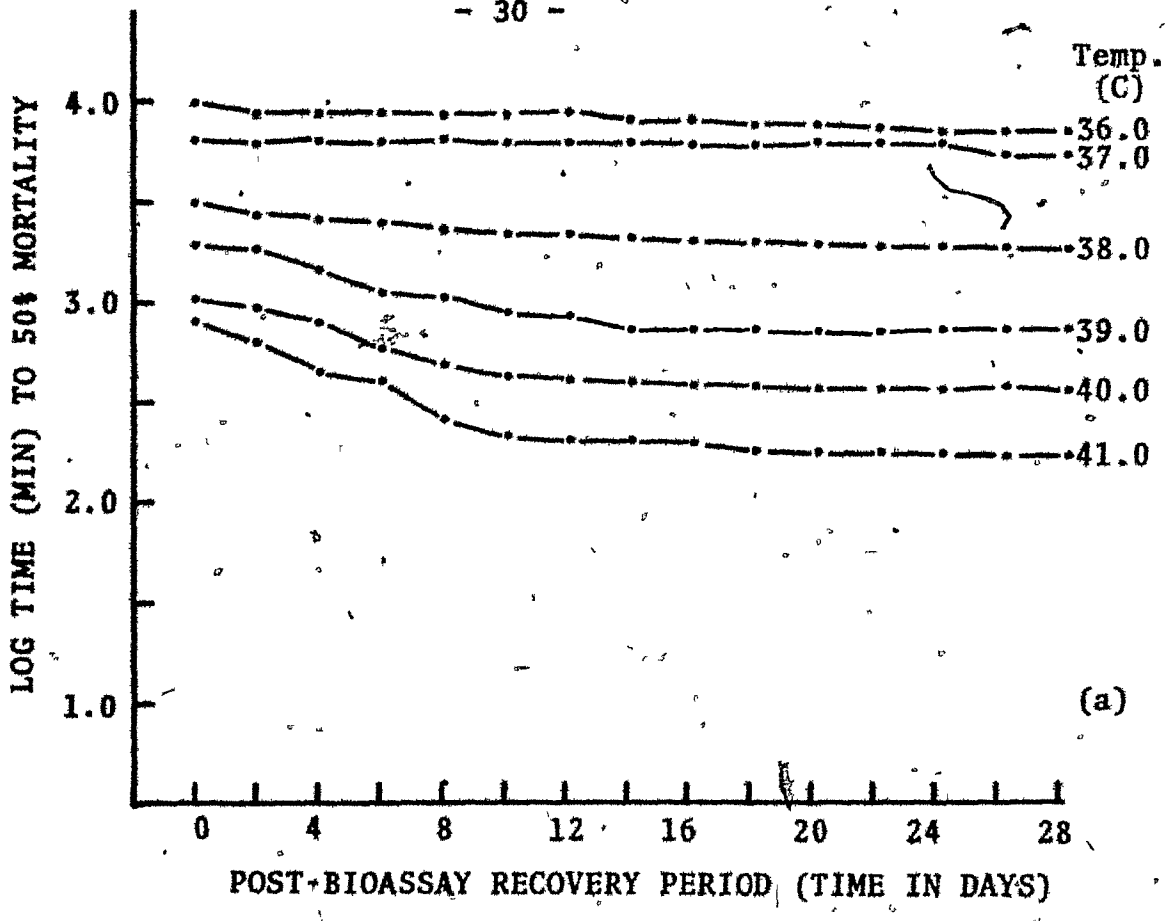

zero-time
28th day
TEST TEMPERATURE (C)
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
34.0
(b)
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY

Figure 8(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Modiolus demissus* acclimated to 15‰ salinity, 15 C and tested at 15‰ salinity.

Figure 8(b) Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Modiolus demissus* acclimated at 15‰ salinity, 15 C and tested at 15‰ salinity.


Temp. (C)
36.0
37.0
38.0
39.0
40.0
41.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

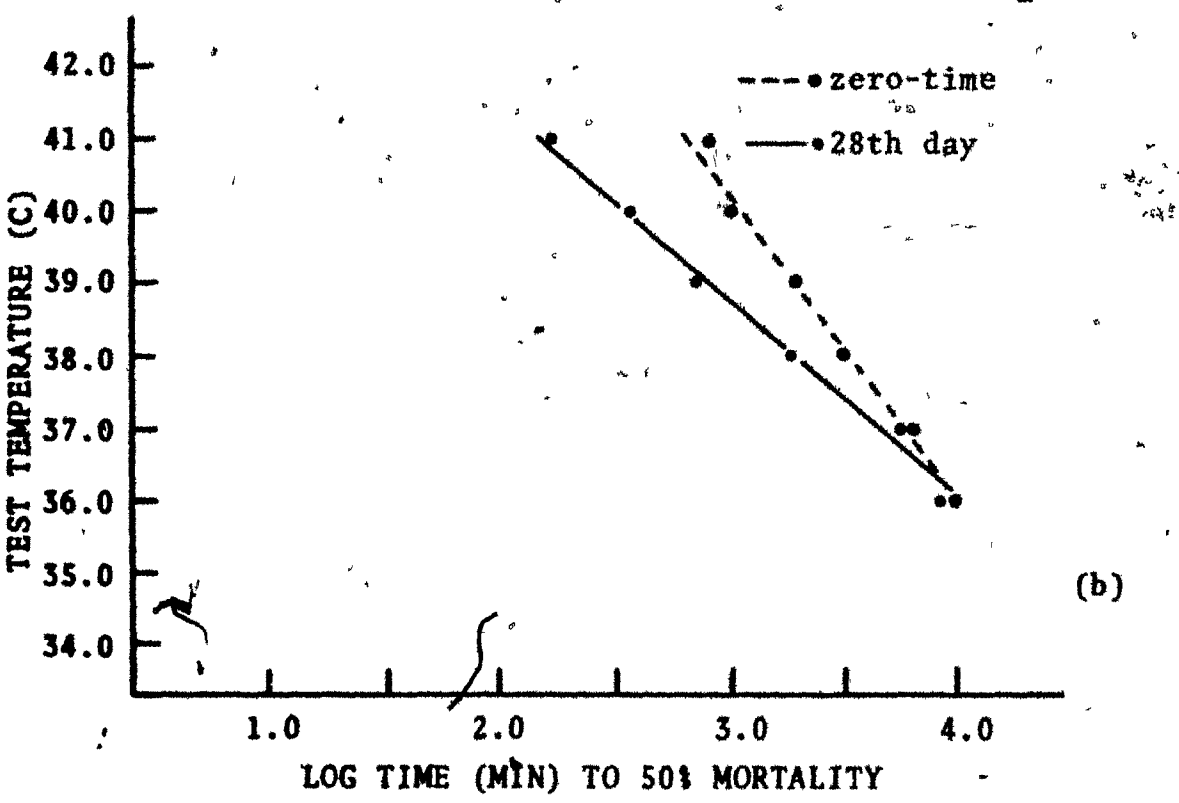
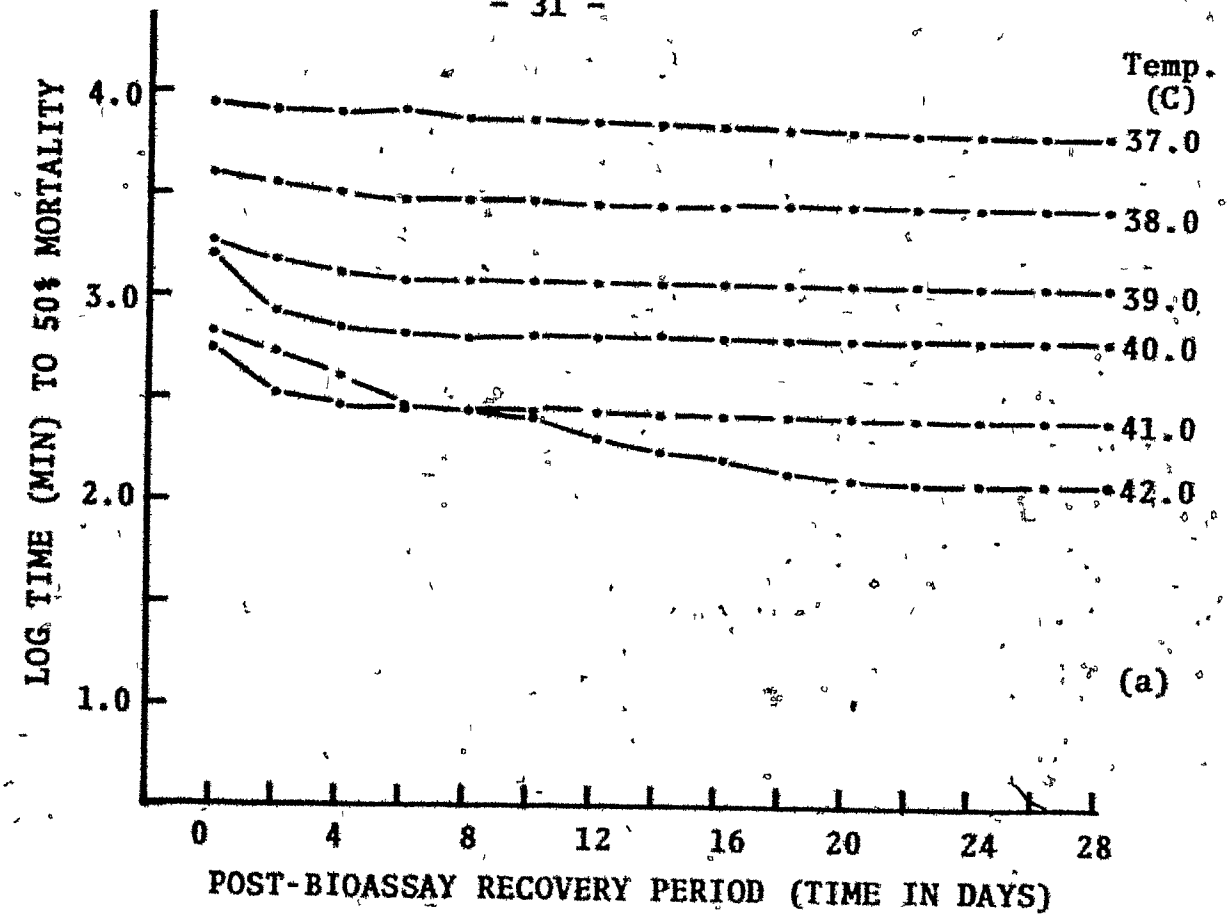

zero-time
28th day
TEST TEMPERATURE (C)
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
34.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 9(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Modiolus demissus* acclimated to 15‰ salinity, 25 C and tested at 15‰ salinity.

Figure 9(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Modiolus demissus* acclimated at 15‰ salinity, 25 C and tested at 15‰ salinity.


Temp. (C)
37.0
38.0
39.0
40.0
41.0
42.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

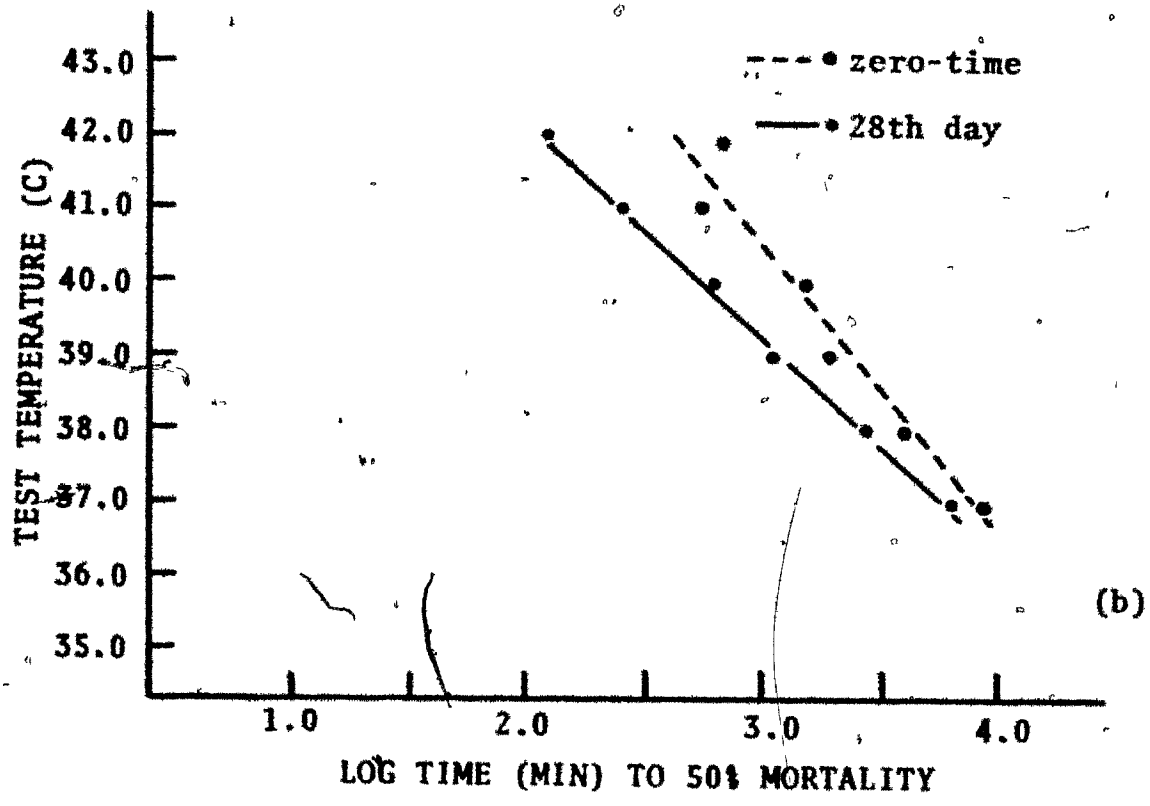
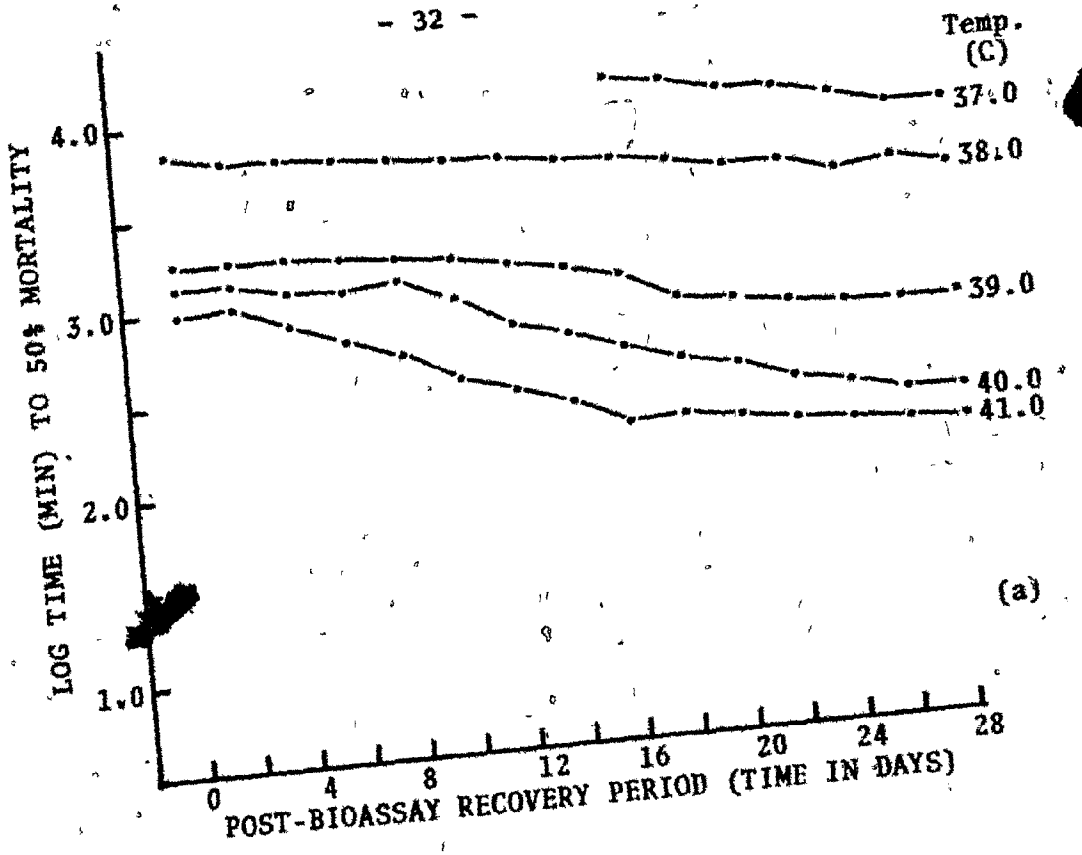

zero-time
28th day
TEST TEMPERATURE (C)
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 10(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Modiolus demissus* acclimated to 30‰ salinity, 5 C and tested at 30‰ salinity.

Figure 10(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Modiolus demissus* acclimated at 30‰ salinity, 15 C and tested at 30‰ salinity.


Temp. (C)
37.0
38.0
39.0
40.0
41.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

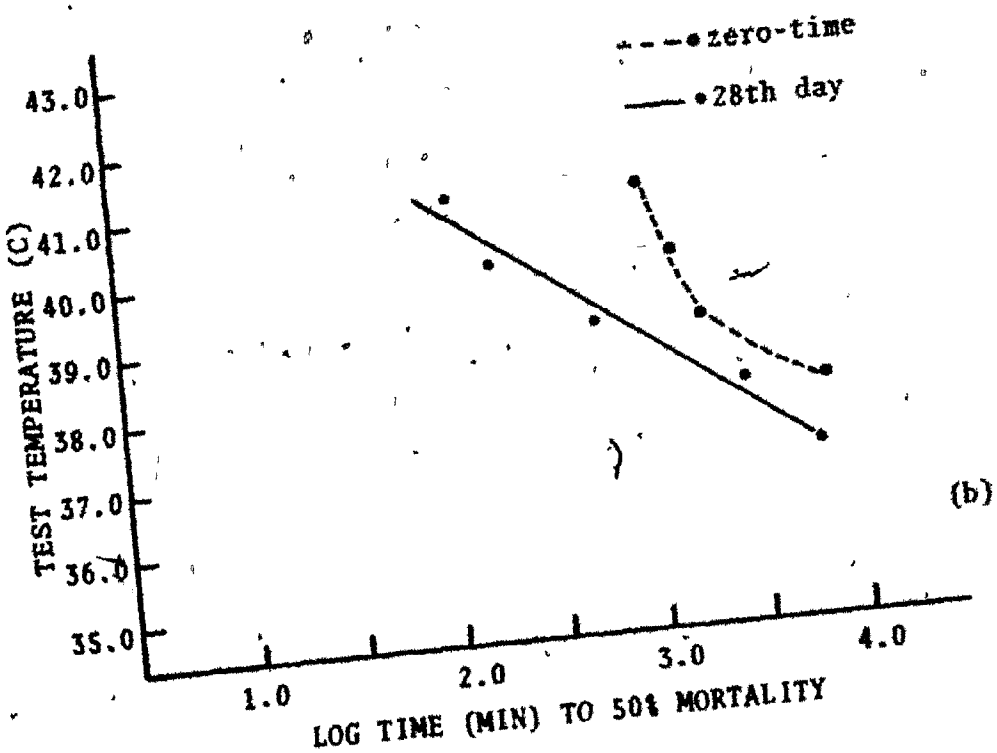
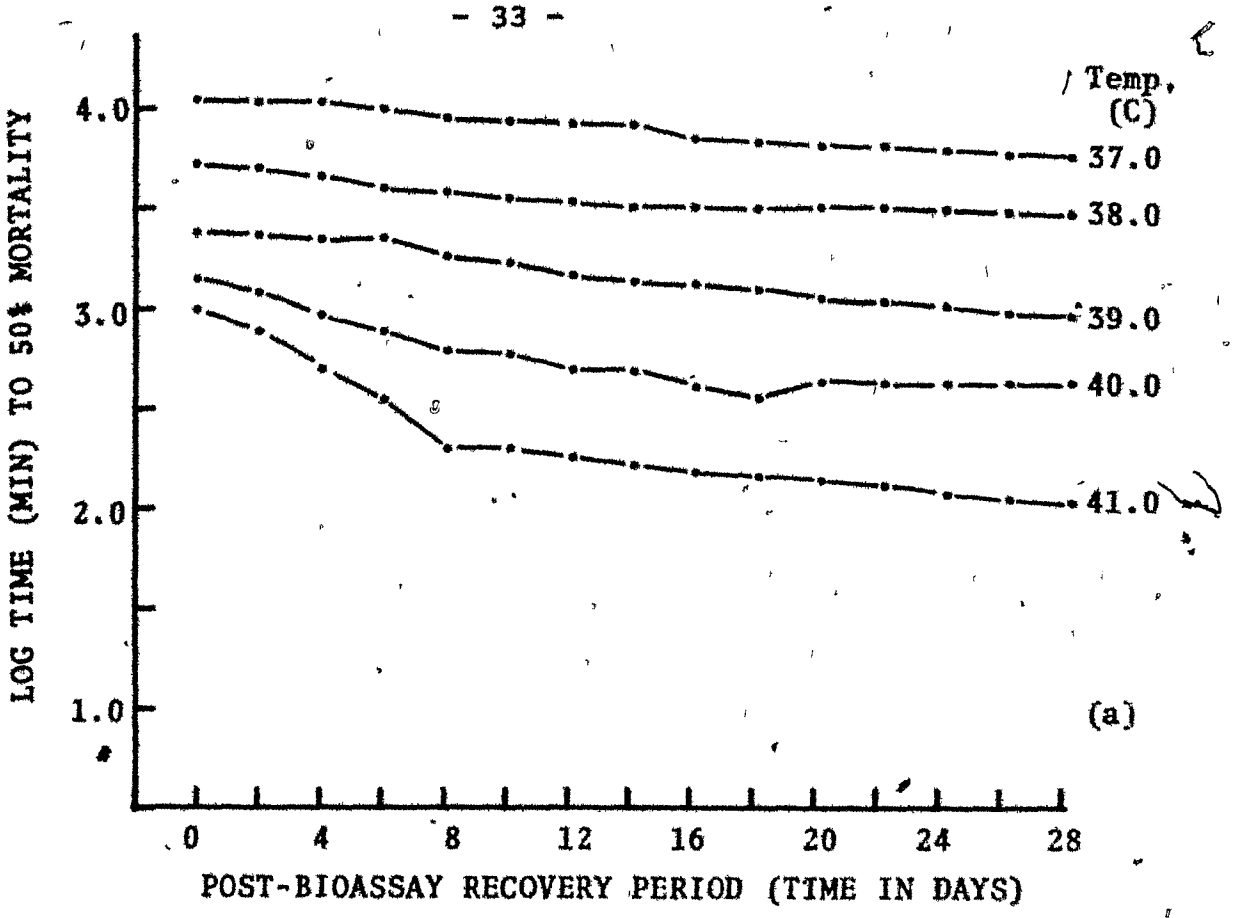

zero-time
28th day
TEST TEMPERATURE (C)
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 11(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Modiolus demissus* acclimated to 30‰ salinity, 15 C and tested at 30‰ salinity.

Figure 11(b) Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Modiolus demissus* acclimated to 30‰ salinity, 15 C and tested at 30‰ salinity.


Temp. (C)
37.0
38.0
39.0
40.0
41.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
(a)
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)

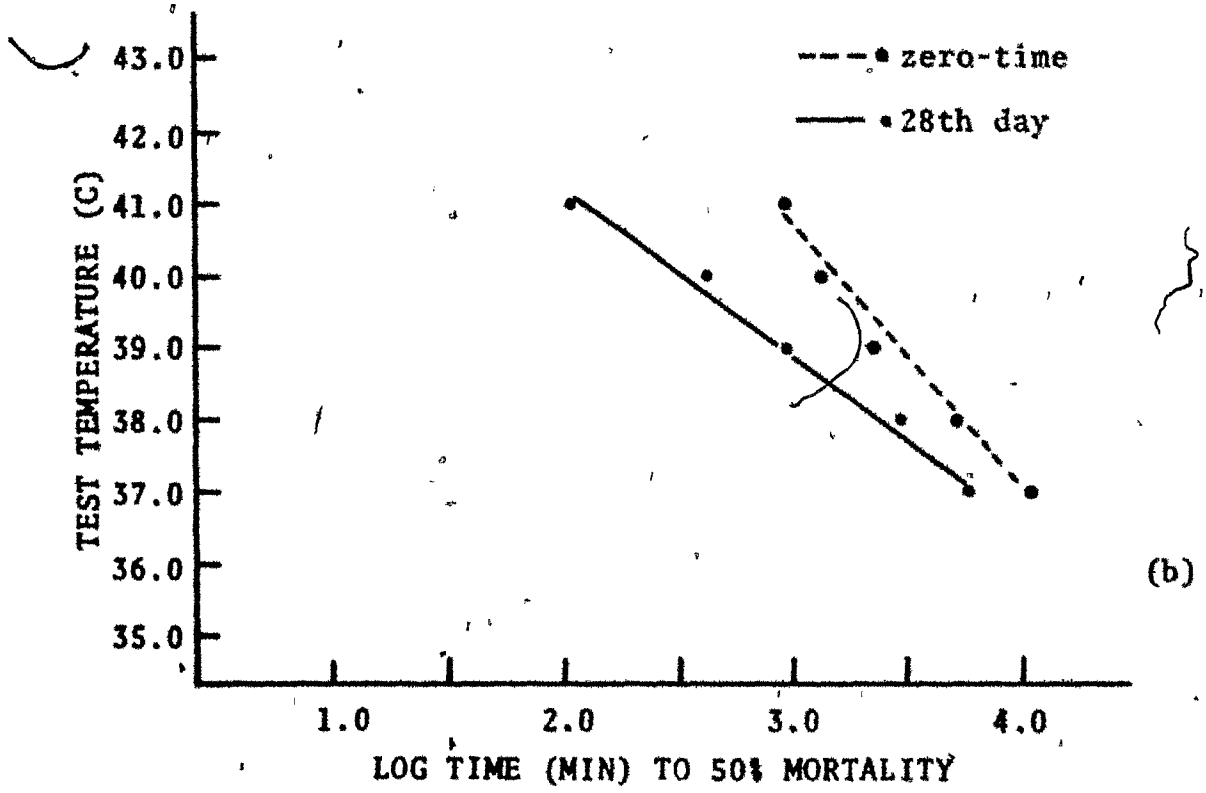
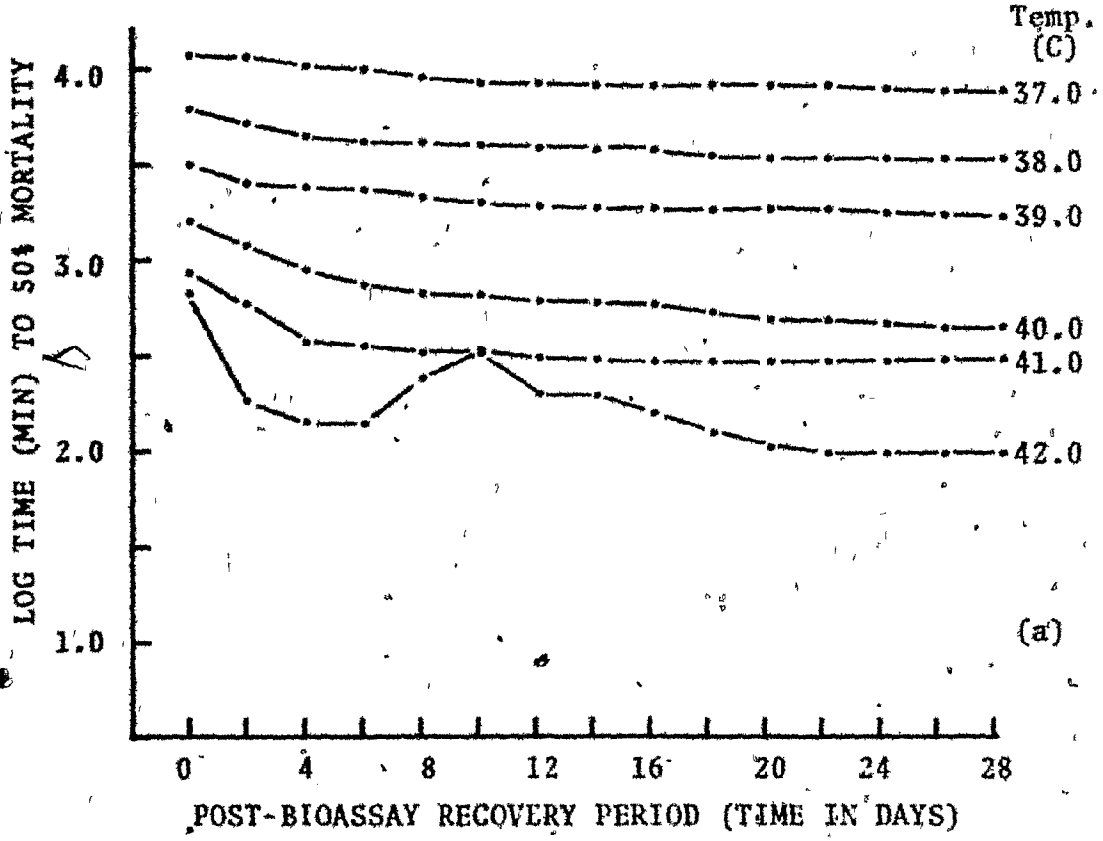

zero-time
28th day
TEST TEMPERATURE (C)
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
(b)
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY

Figure 12(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Modiolus demissus* acclimated at 30‰ salinity, 25 C and tested at 30‰ salinity.

Figure 12(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Modiolus demissus* acclimated at 30‰ salinity, 25 C and tested at 30‰ salinity.


Temp. (C)
37.0
38.0
39.0
40.0
41.0
42.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
(a)
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)

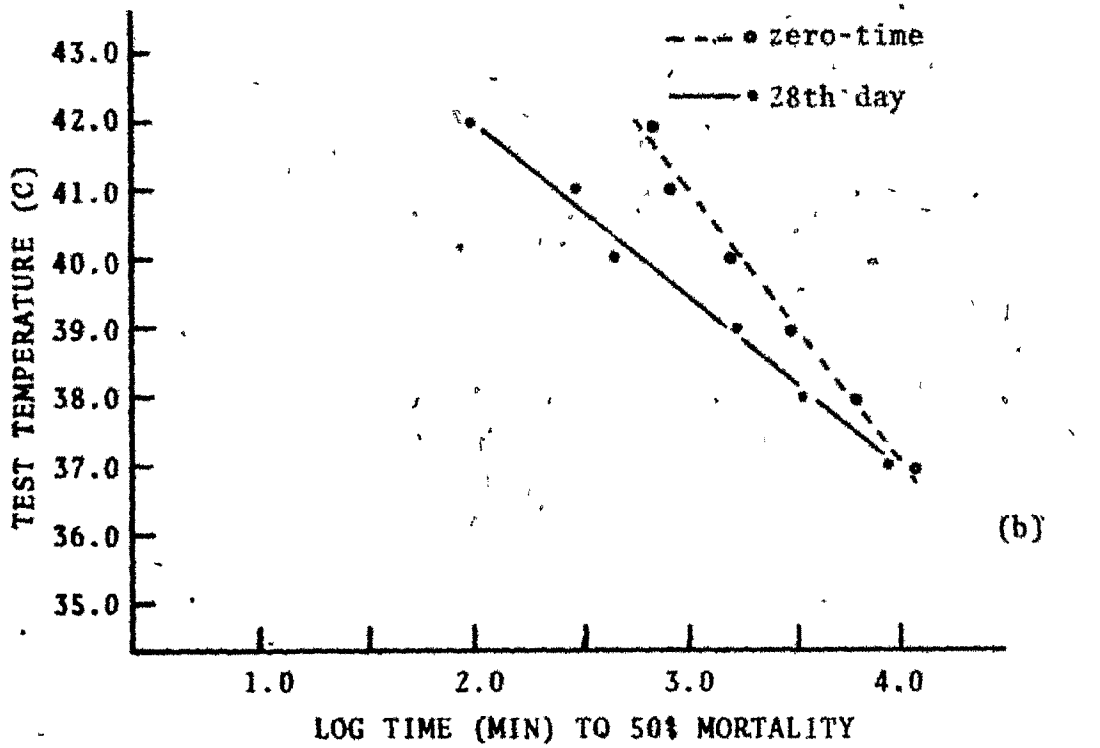
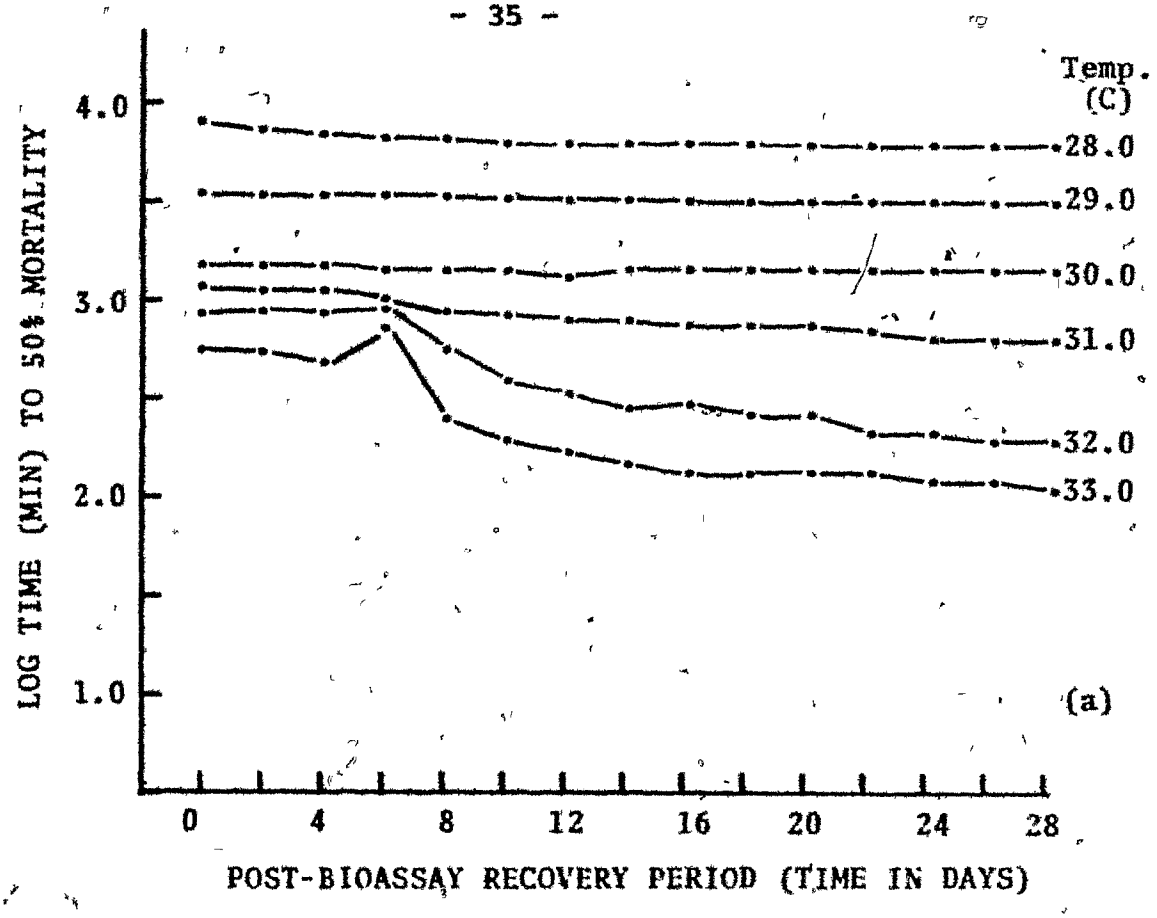

zero-time
28th day
TEST TEMPERATURE (C)
43.0
42.0
41.0
40.0
39.0
38.0
37.0
36.0
35.0
(b)
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY

Figure 13(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mya arenaria* acclimated to 15‰ salinity, 5 C and tested at 15‰ salinity.

Figure 13(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mya arenaria* acclimated at 15‰ salinity, 5 C and tested at 15‰ salinity.


Temp. (C)
28.0
29.0
30.0
31.0
32.0
33.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

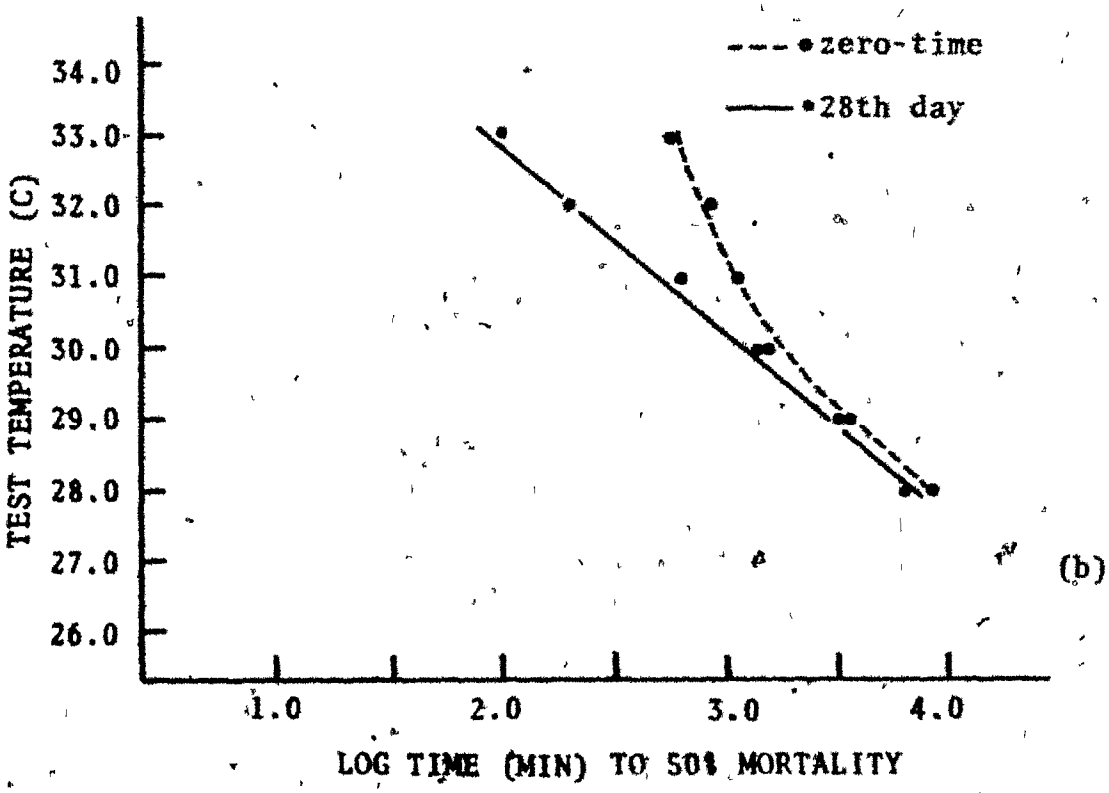
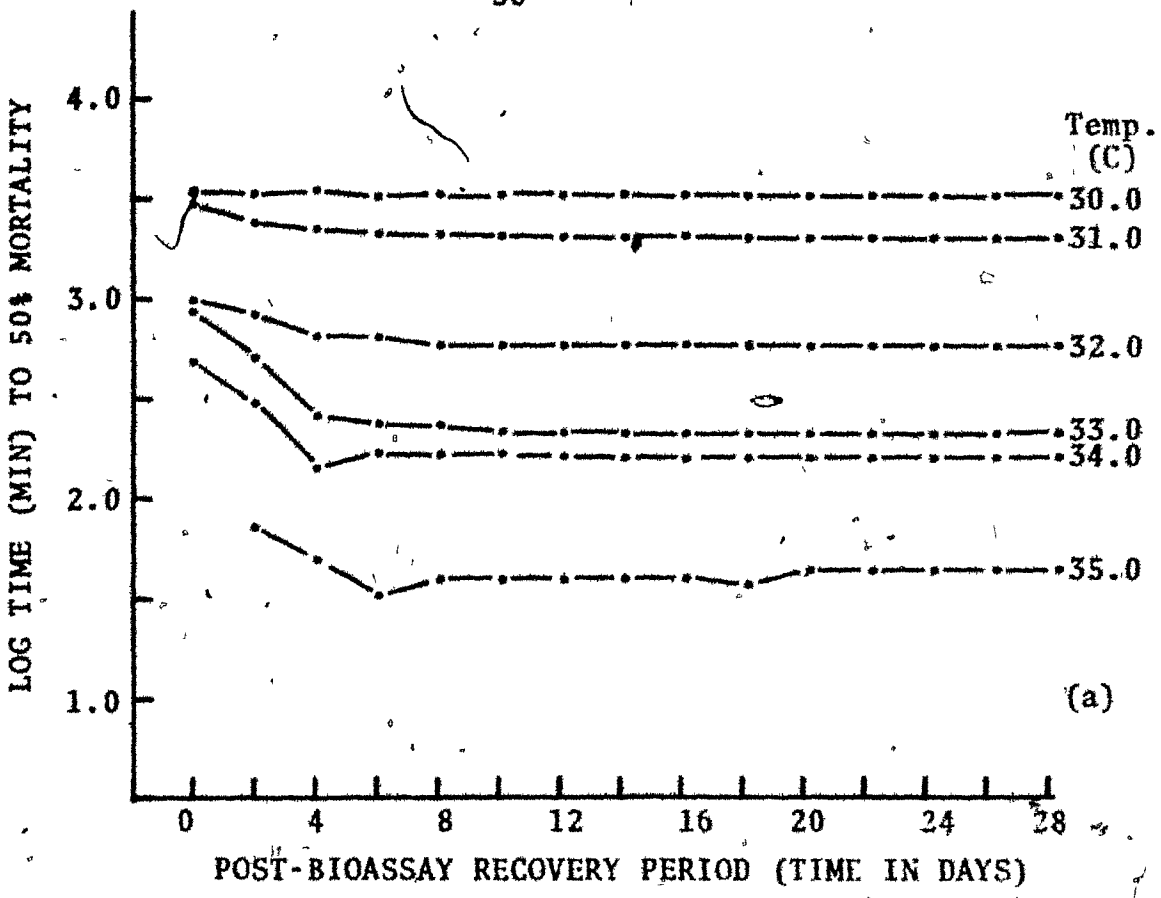

zero-time
28th day
TEST TEMPERATURE (C)
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 14(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mya arenaria* acclimated to 15‰ salinity, 15 C and tested at 15‰ salinity.

Figure 14(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mya arenaria* acclimated at 15‰ salinity, 25 C and tested at 15‰ salinity.


LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
Temp. (C)
30.0
31.0
32.0
33.0
34.0
35.0
(a)
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)

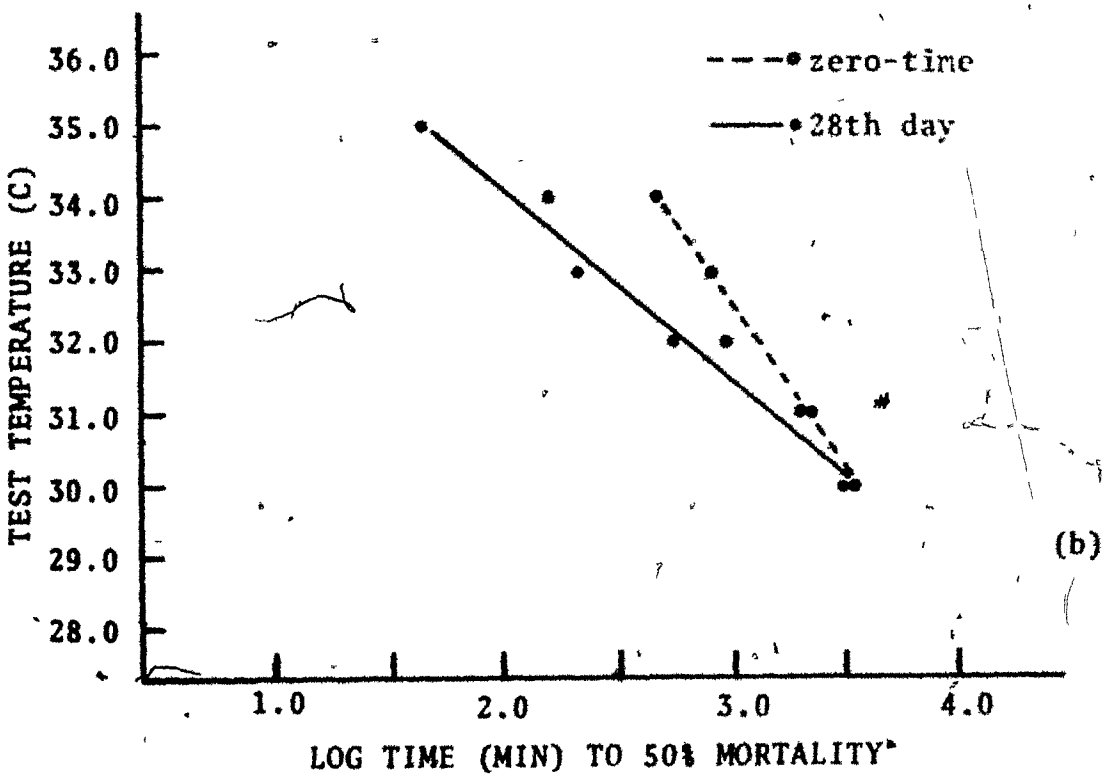
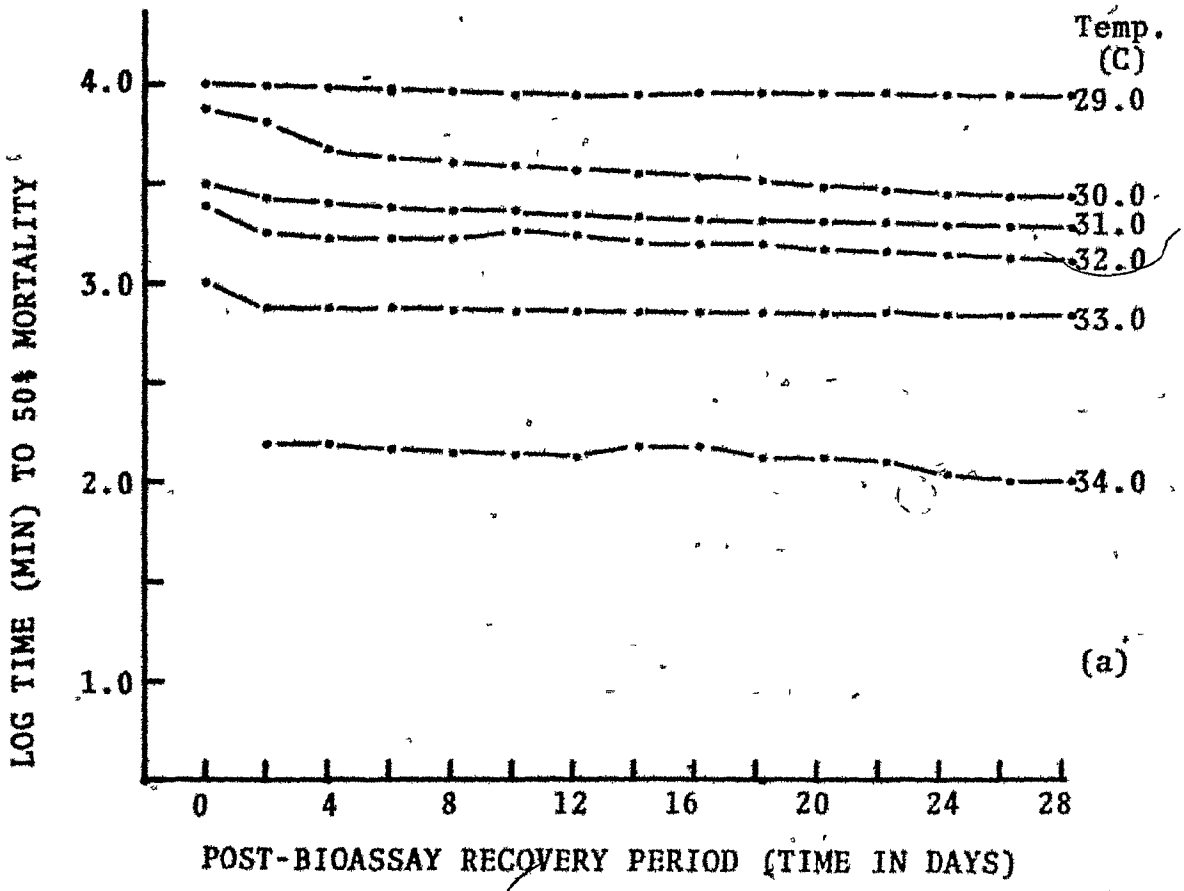

TEST TEMPERATURE (C)
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
zero-time
28th day
(b)
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY

Figure 15(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mya arenaria* acclimated to 15‰ salinity, 25 C and tested at 15‰ salinity.

Figure 15(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mya arenaria* acclimated at 15‰ salinity, 25 C and tested at 15‰ salinity.


Temp. (C)
29.0
30.0
31.0
32.0
33.0
34.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

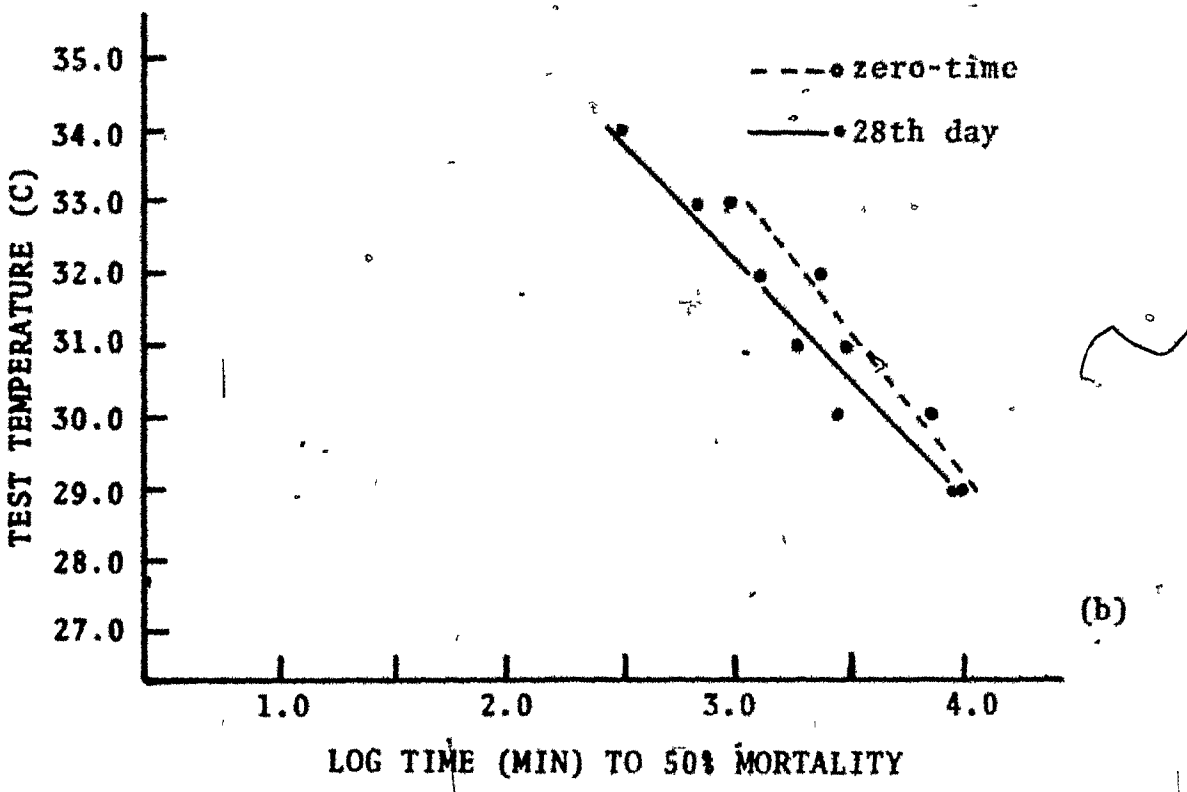
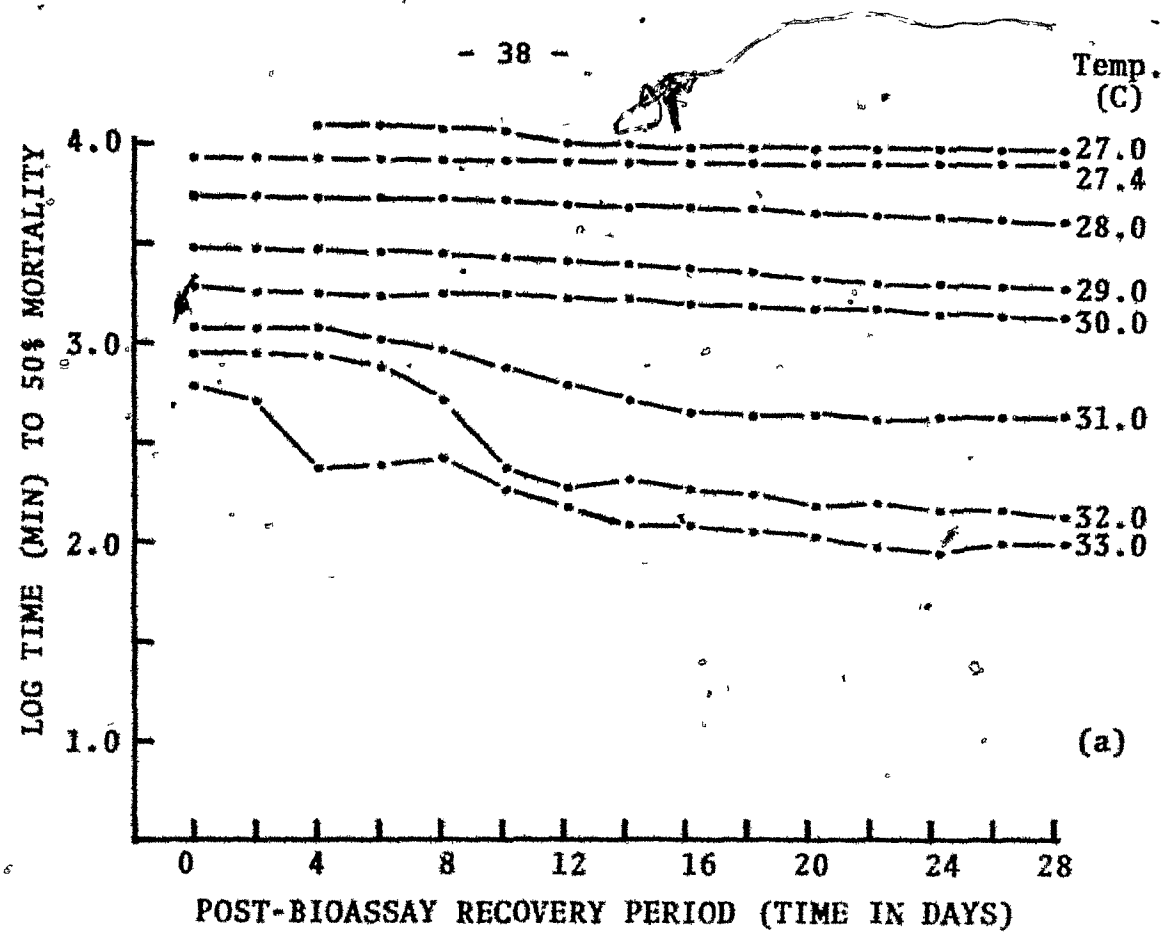

zero-time
28th day
TEST TEMPERATURE (C)
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 16(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mya arenaria* acclimated to 30‰ salinity, 5°C and tested at 30‰ salinity.

Figure 16(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mya arenaria* acclimated at 30‰ salinity, 5 C and tested at 30‰ salinity.


Temp. (C)
27.0
27.4
28.0
29.0
30.0
31.0
32.0
33.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

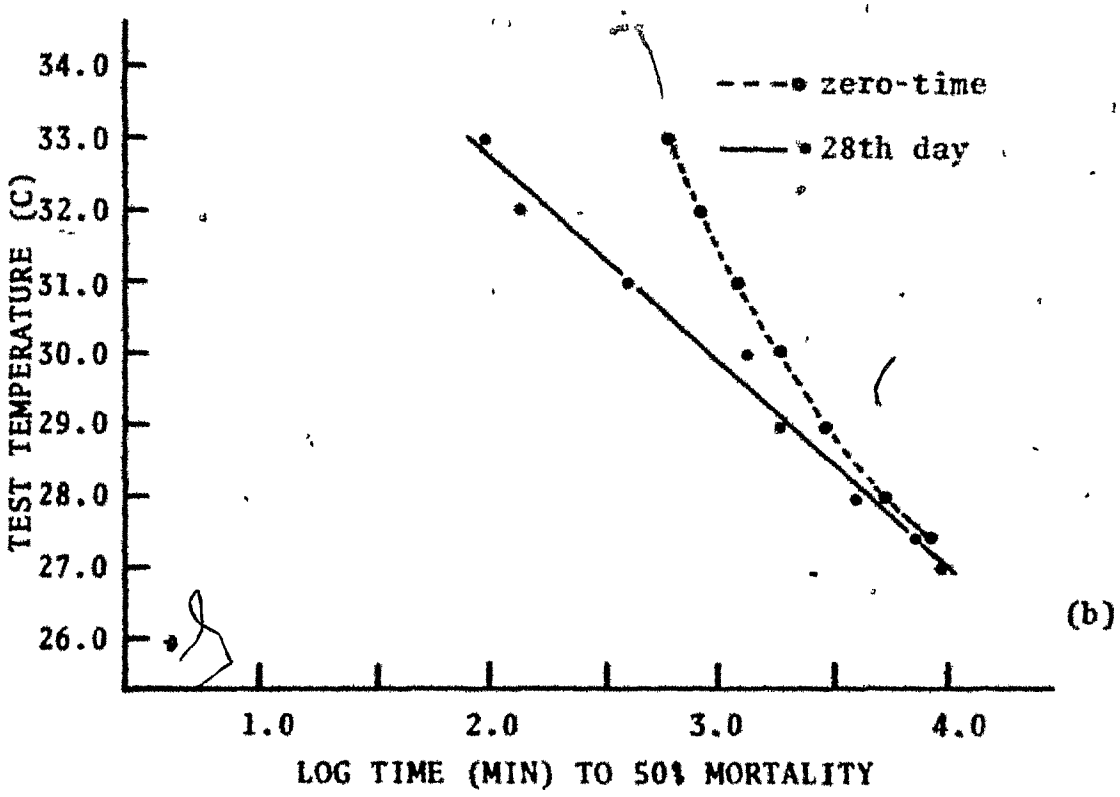

zero-time
28th day
TEST TEMPERATURE (C)
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 17(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mya arenaria* acclimated to 30‰ salinity, 15 C and tested at 30‰ salinity.

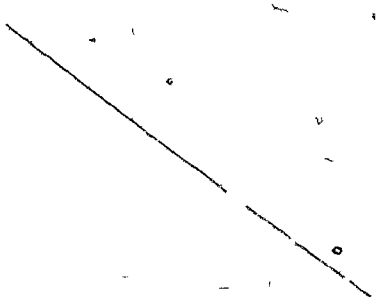
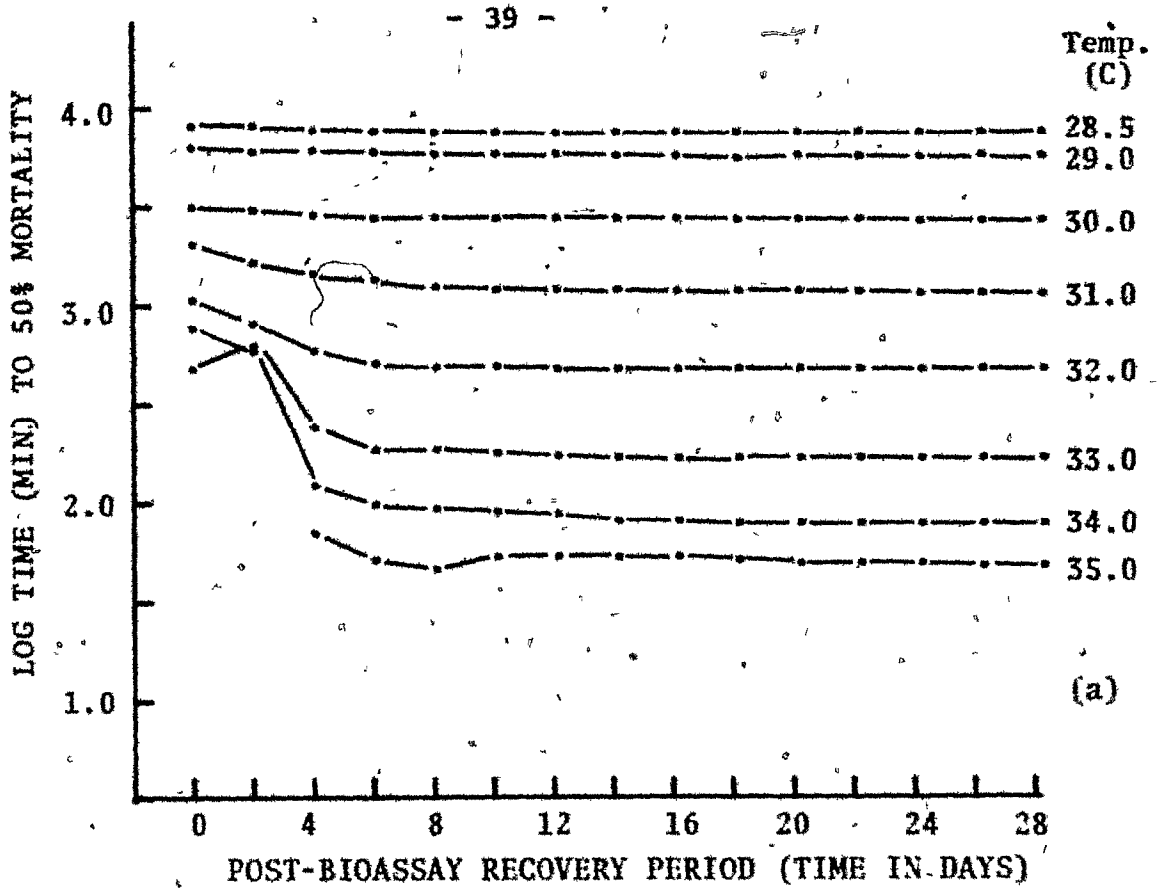


Figure 17(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mya arenaria* acclimated at 30‰ salinity, 15 C and tested at 30‰ salinity.


Temp. (C)
28.5
29.0
30.0
31.0
32.0
33.0
34.0
35.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

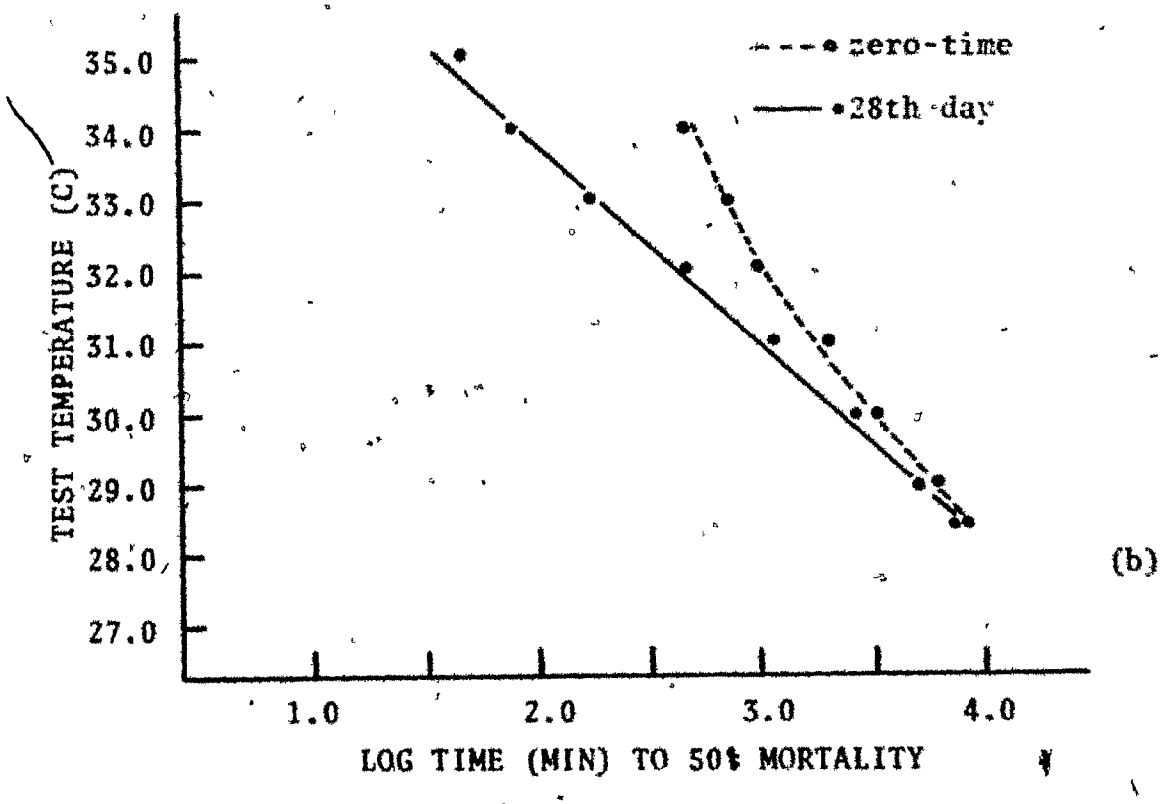
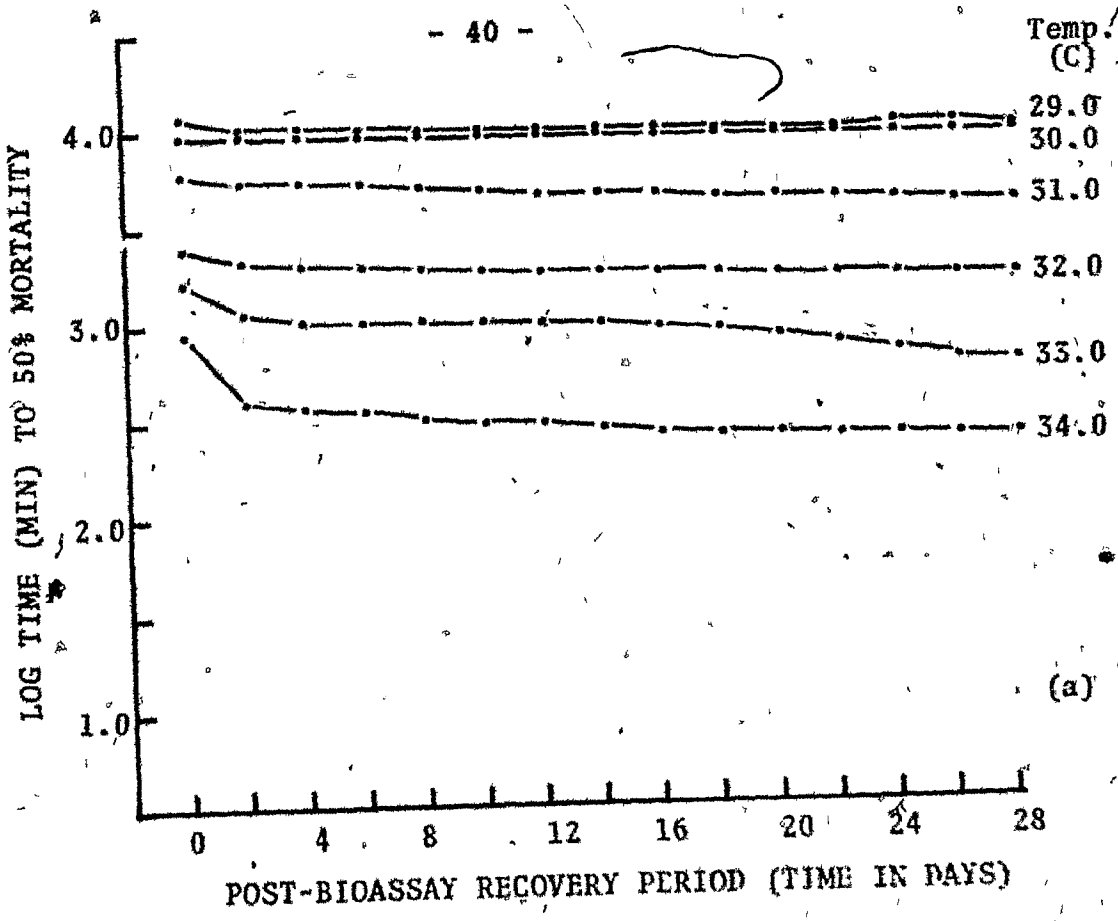

zero-time
28th day
TEST TEMPERATURE (C)
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 18(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mya arenaria* acclimated to 30‰ salinity, 25 C and tested at 30‰ salinity.

Figure 18(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mya arenaria* acclimated at 30‰ salinity, 25 C and tested at 30‰ salinity.


Temp. (C)
29.0
30.0
31.0
32.0
33.0
34.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

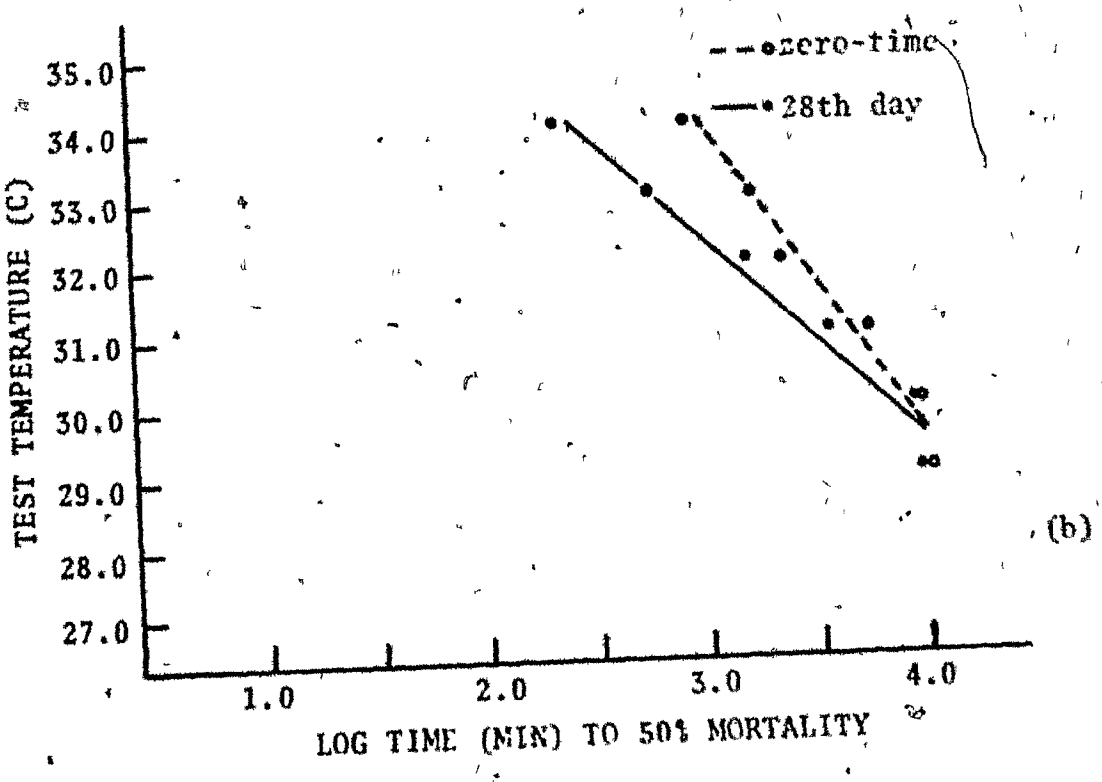
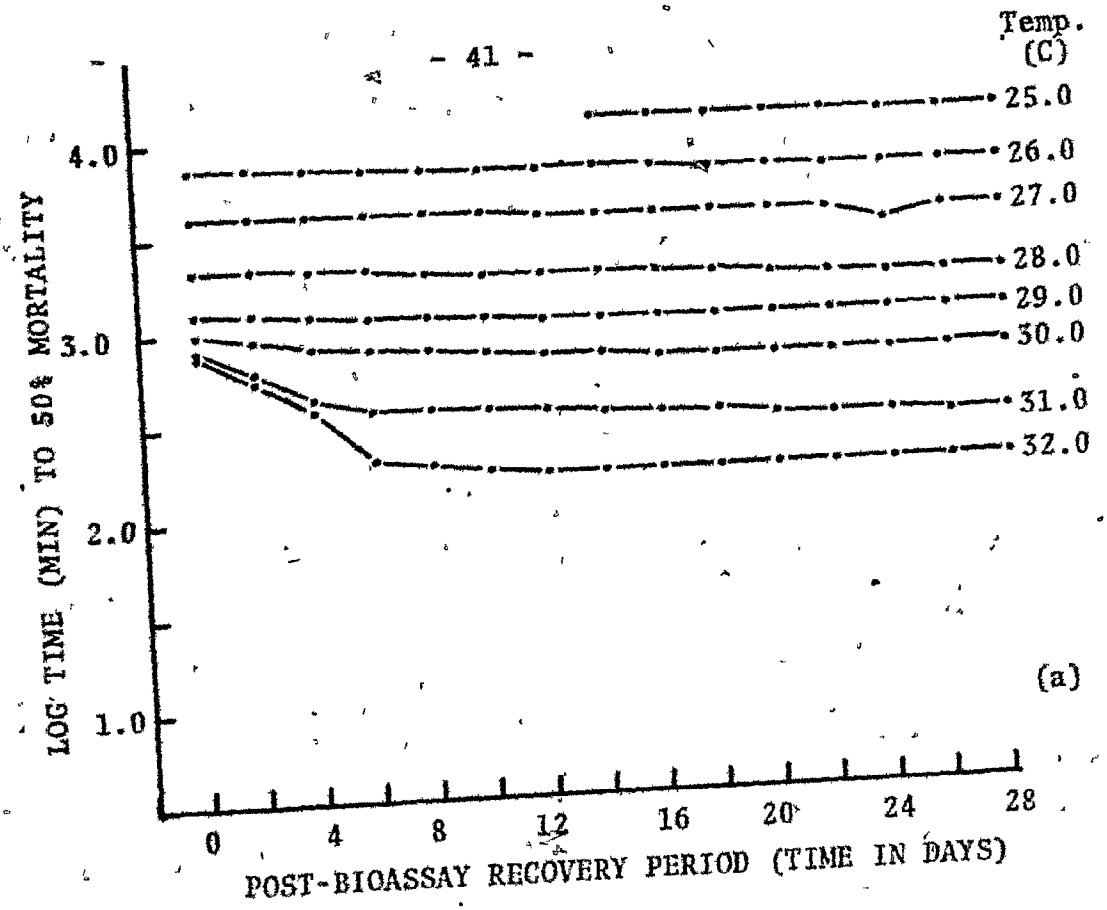

zero-time
28th day
TEST TEMPERATURE (C)
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 19(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mytilus edulis* acclimated to 15‰ salinity, 5 C and tested at 15‰ salinity.

Figure 19(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mytilus edulis* acclimated at 15‰ salinity, 5 C and tested at 15‰ salinity.


Temp. (C)
25.0
26.0
27.0
28.0
29.0
30.0
31.0
32.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

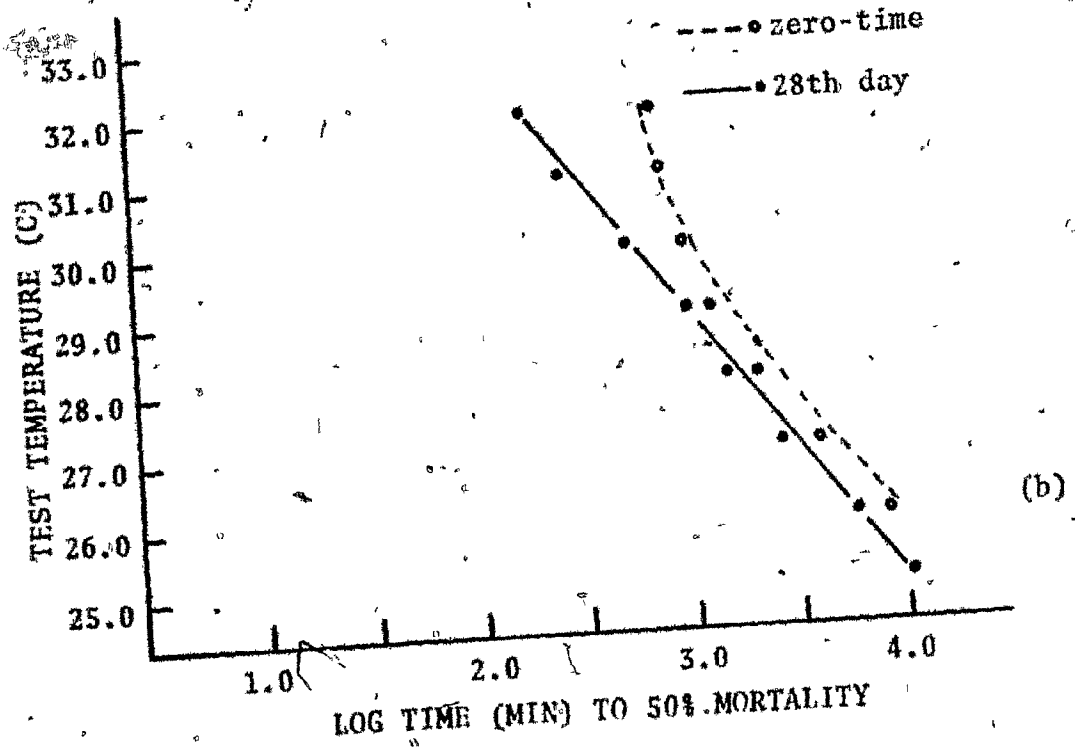
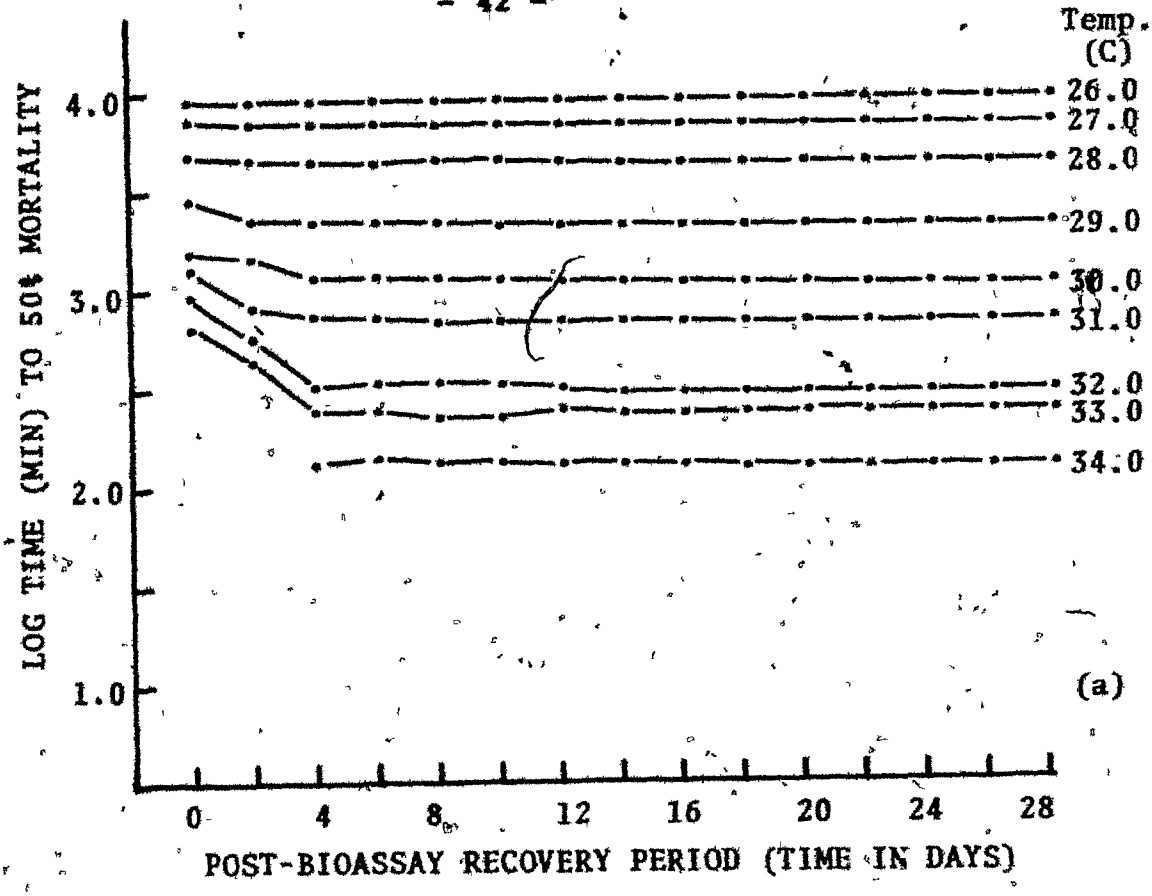
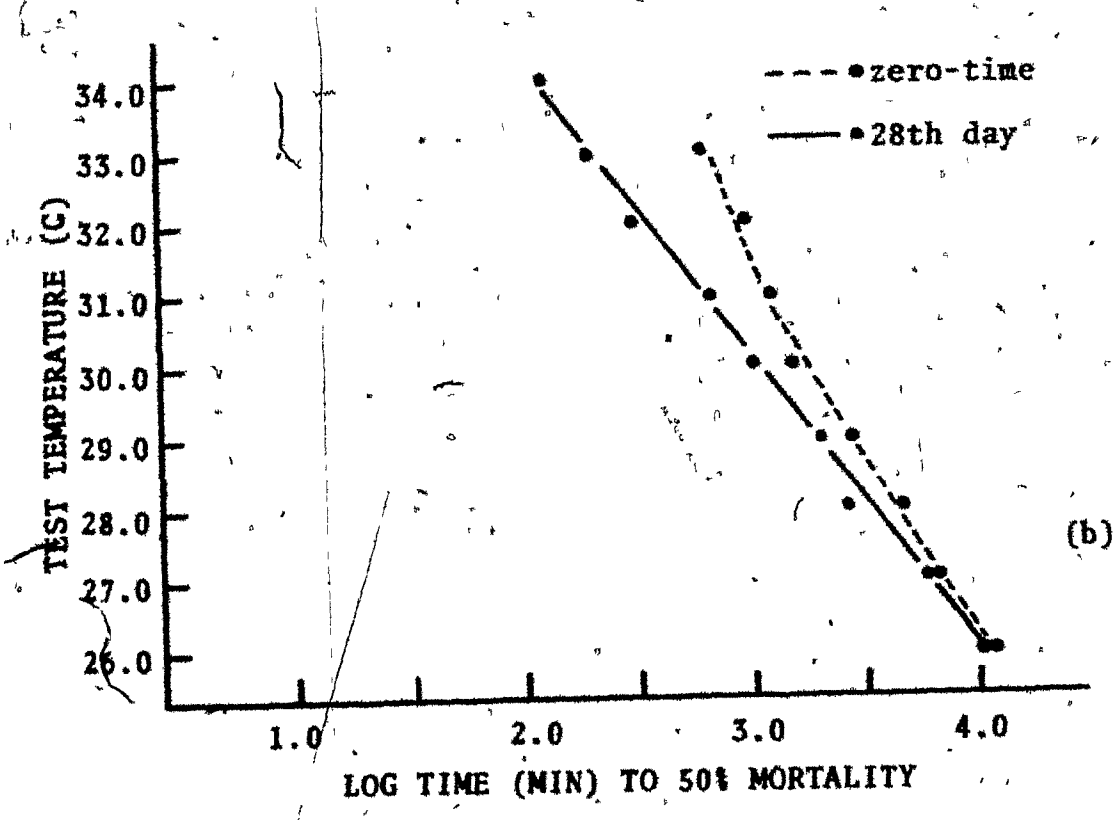

zero-time
28th day
TEST TEMPERATURE (C)
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 20(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mytilus edulis* acclimated to 15‰ salinity, 15 C and tested at 15‰ salinity.

Figure 20(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mytilus edulis* acclimated at 15‰ salinity, 15 C and tested at 15‰ salinity.


Temp. (C)
26.0
27.0
28.0
29.0
30.0
31.0
32.0
33.0
34.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
(a)
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)


zero-time
28th day
TEST TEMPERATURE (C)
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
(b)
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY

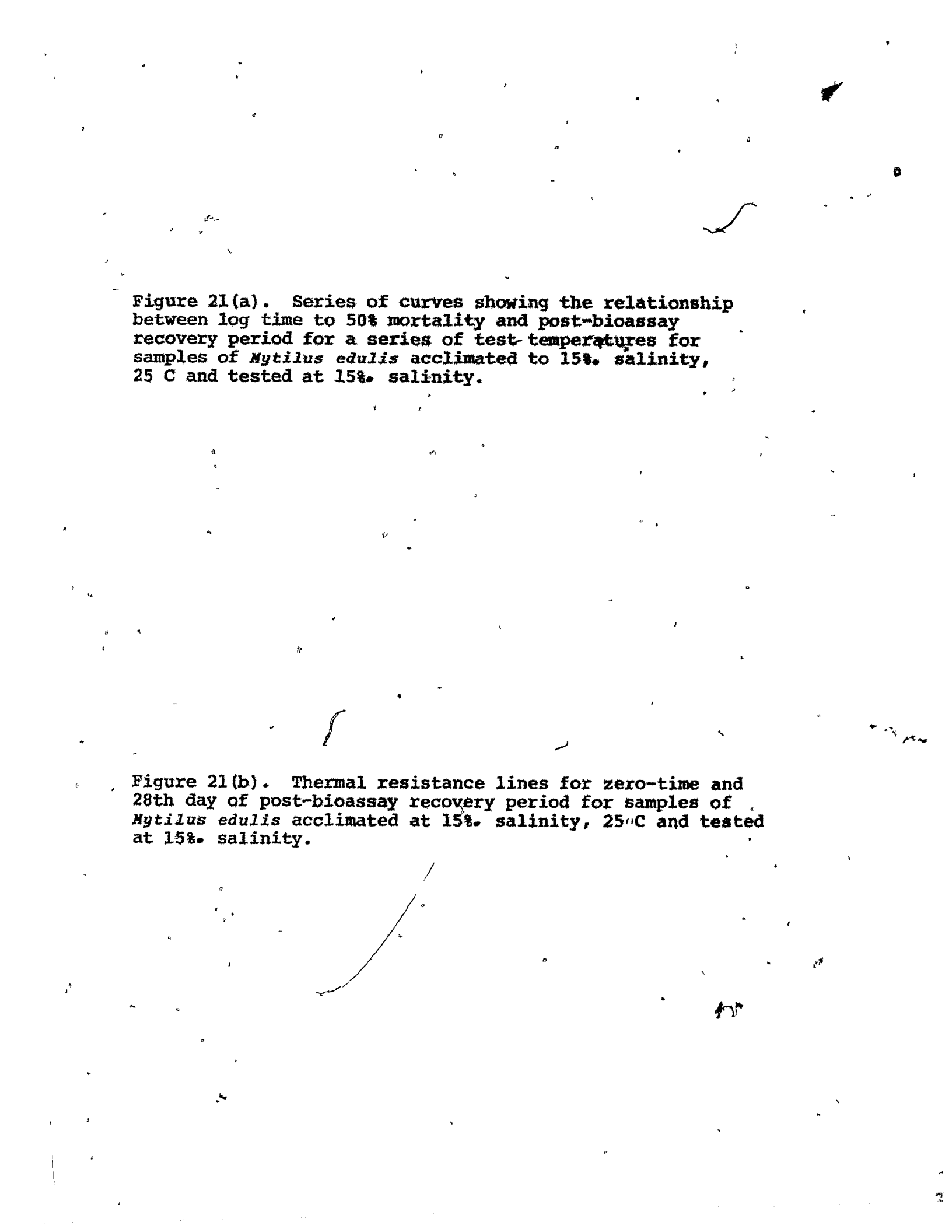
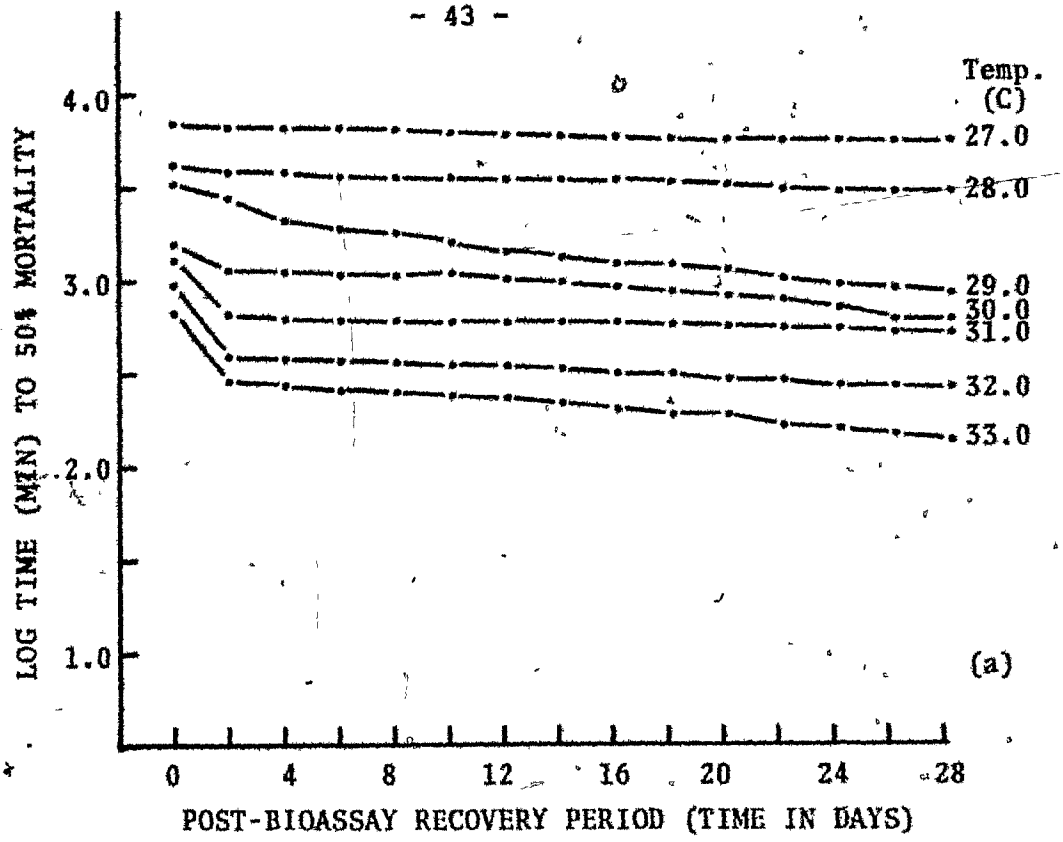


Figure 21(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mytilus edulis* acclimated to 15‰ salinity, 25 C and tested at 15‰ salinity.

Figure 21(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mytilus edulis* acclimated at 15‰ salinity, 25°C and tested at 15‰ salinity.


Temp. (C)
27.0
28.0
29.0
30.0
31.0
32.0
33.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

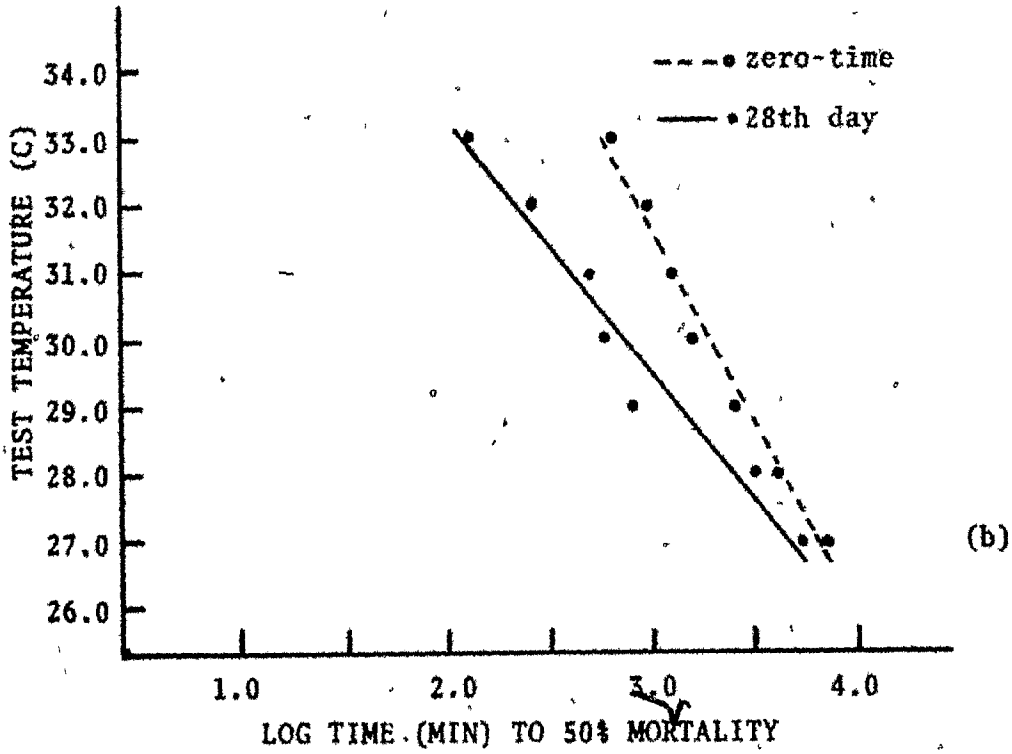
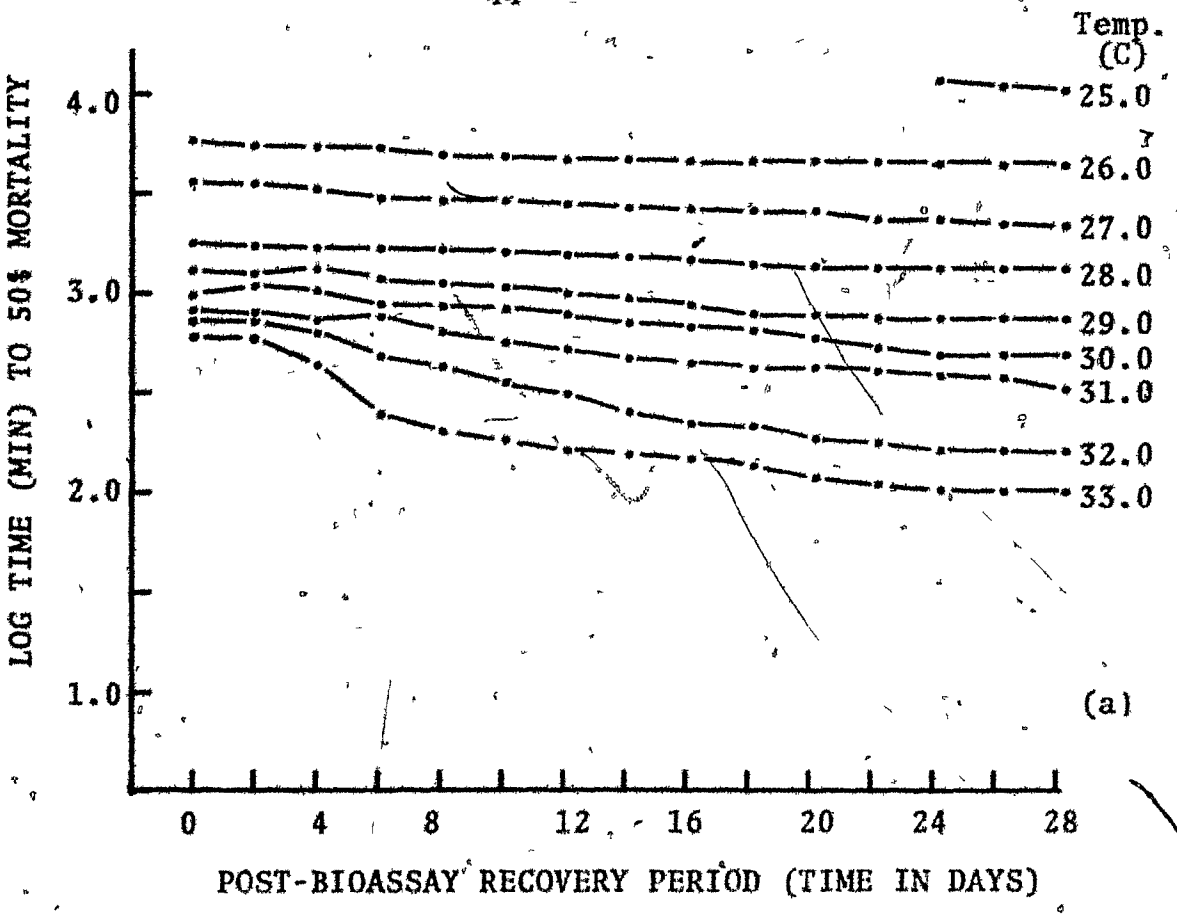

zero-time
28th day
TEST TEMPERATURE (C)
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 22(a) Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mytilus edulis* acclimated to 30‰ salinity, 5 C and tested at 30‰ salinity.

Figure 22(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mytilus edulis* acclimated at 30‰ salinity, 5 C and tested at 30‰ salinity.


Temp. (C)
25.0
26.0
27.0
28.0
29.0
30.0
31.0
32.0
33.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

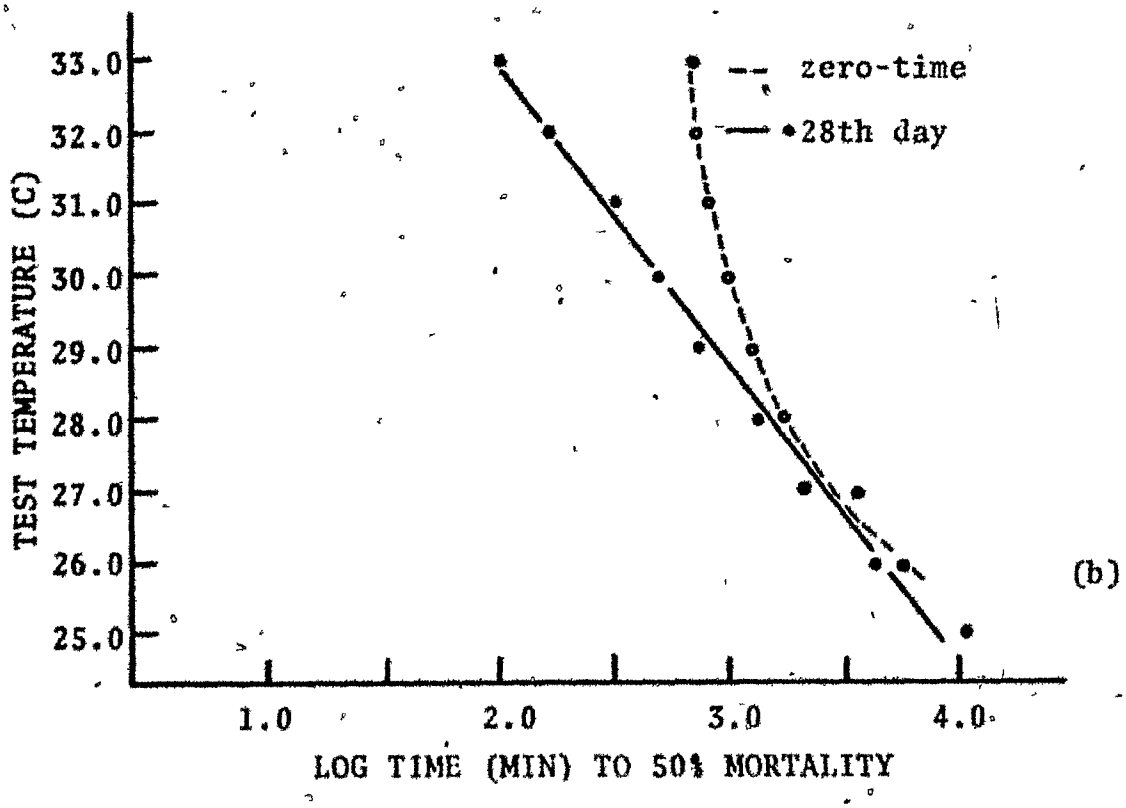
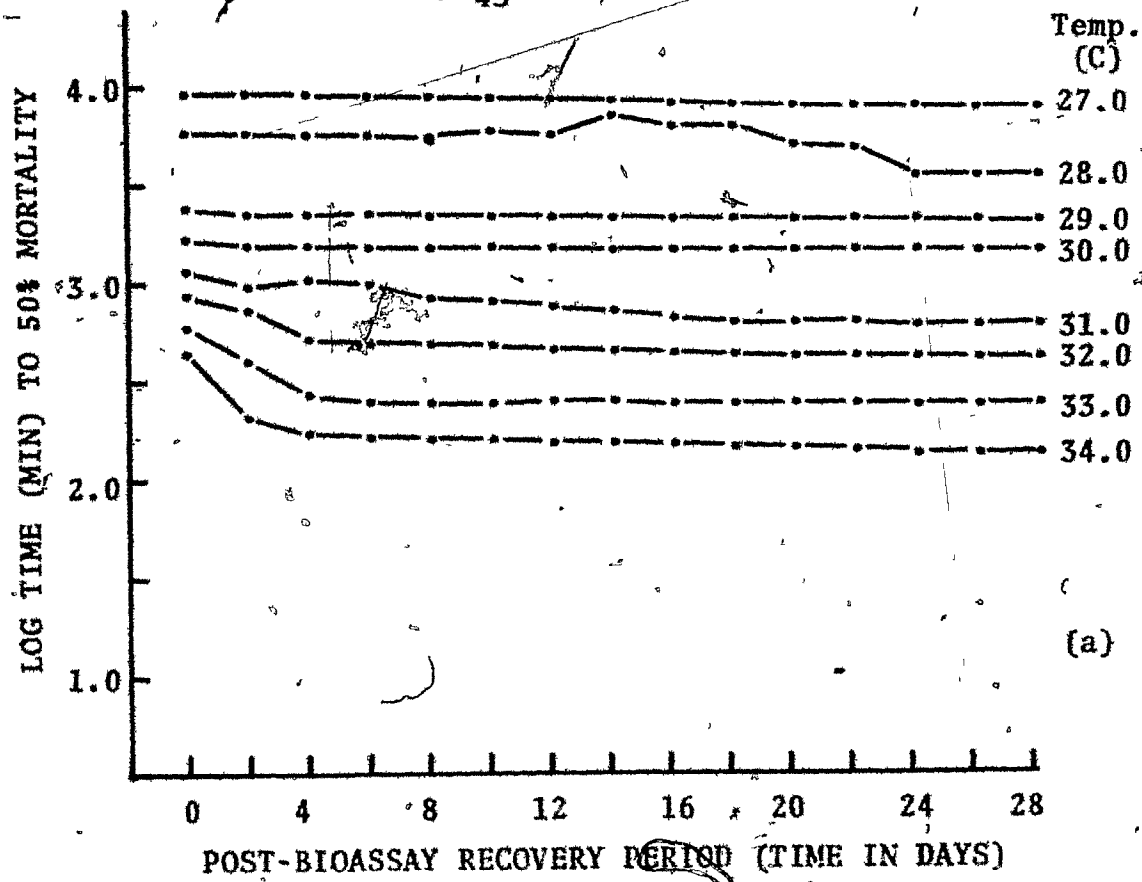

zero-time
28th day
TEST TEMPERATURE (C)
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 23(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mytilus edulis* acclimated to 30‰ salinity, 15 C and tested at 30‰ salinity.

Figure 23 (b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mytilus edulis* acclimated at 30‰ salinity, 15 C and tested at 30‰ salinity.


Temp. (C)
27.0
28.0
29.0
30.0
31.0
32.0
33.0
34.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)

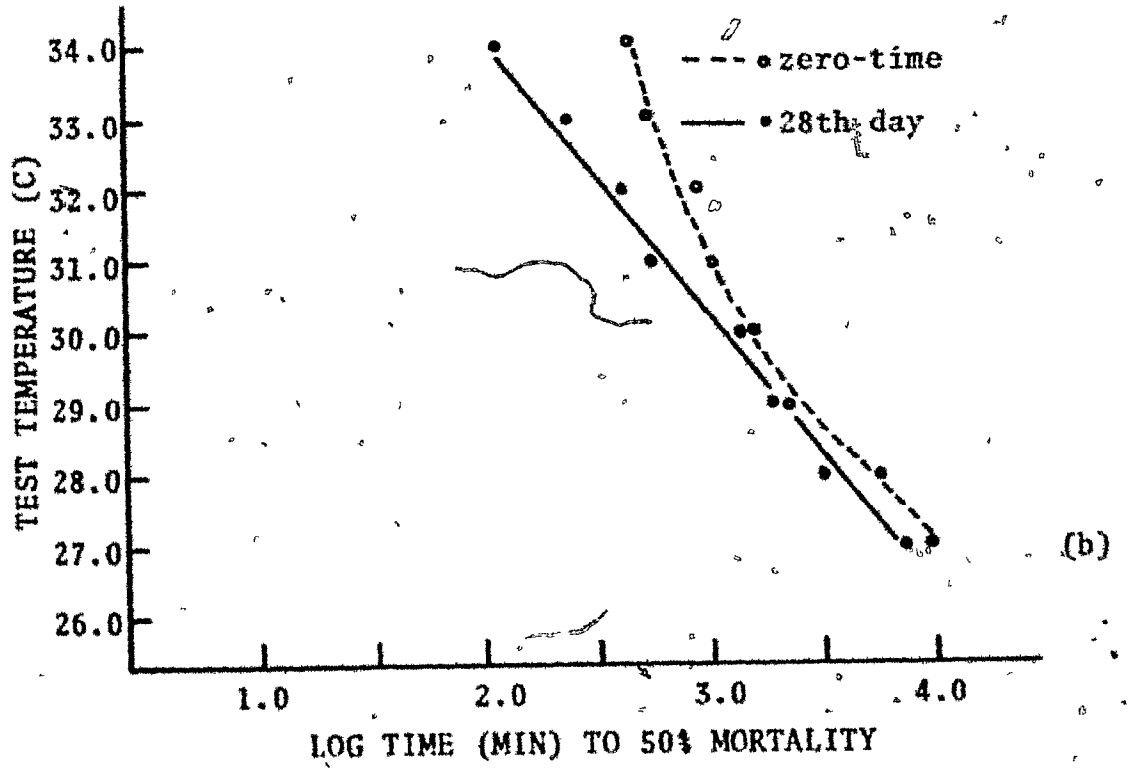
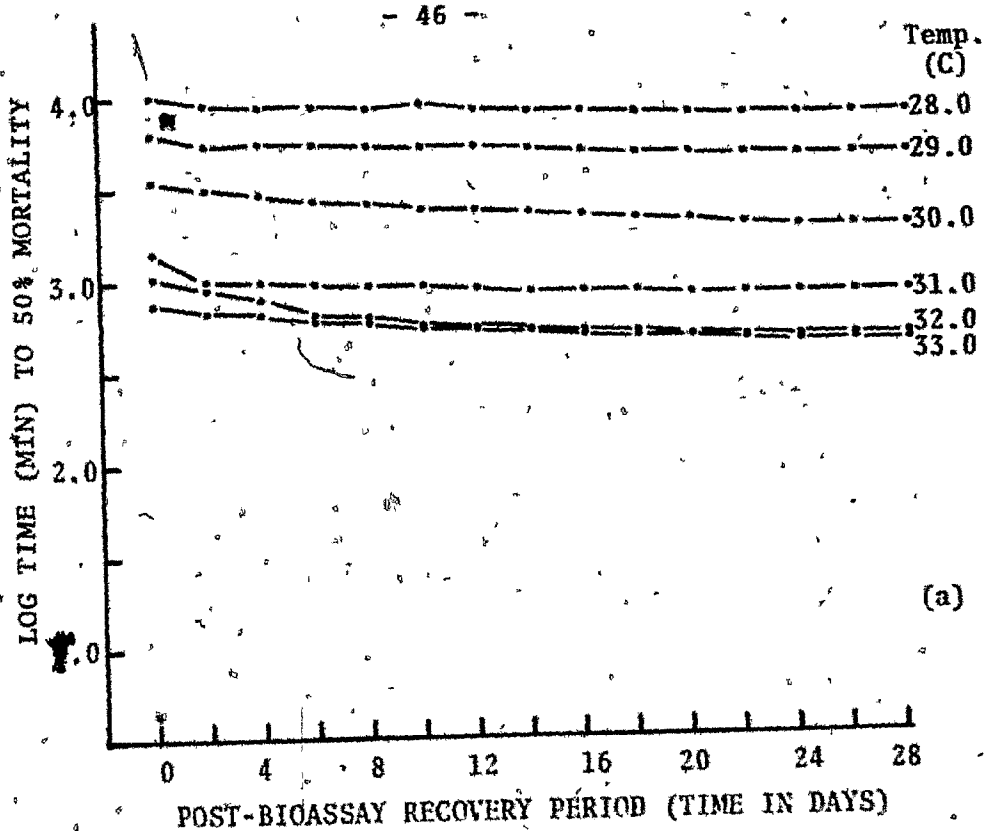
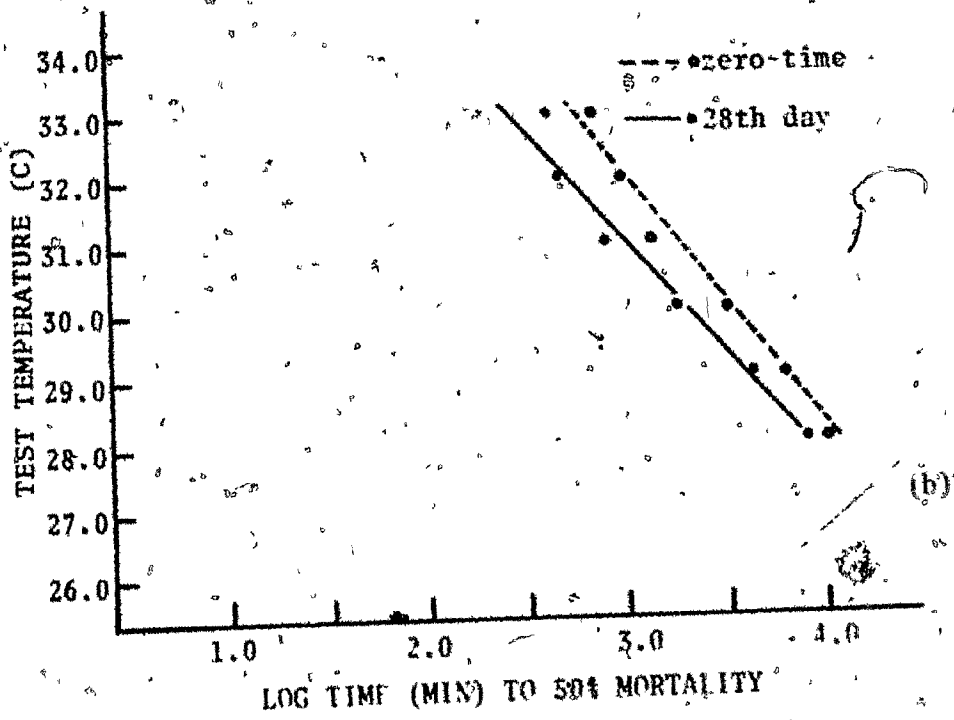

zero-time
28th day
TEST TEMPERATURE (C)
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

Figure 24(a). Series of curves showing the relationship between log time to 50% mortality and post-bioassay recovery period for a series of test temperatures for samples of *Mytilus edulis* acclimated to 30‰ salinity, 25 C and tested at 30‰ salinity.

Figure 24(b). Thermal resistance lines for zero-time and 28th day of post-bioassay recovery period for samples of *Mytilus edulis* acclimated at 30‰ salinity, 25 C and tested at 30‰ salinity.


Temp. (C)
28.0
29.0
30.0
31.0
32.0
33.0
LOG TIME (MIN) TO 50% MORTALITY
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
POST-BIOASSAY RECOVERY PERIOD (TIME IN DAYS)
(a)


zero-time
28th day
TEST TEMPERATURE (C)
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
(b)

analysis was performed on all polynomial equations that were obtained. The second order polynomial equation was considered to provide a more adequate fit to the data if the F-value was significant and if the sum of squares due to deviation about the regression was less than that obtained for the first order polynomial. Table 1 indicates the results of the above procedure.

All subsequent statistical analyses were performed on the thermal resistance lines obtained from the data for the 28th day of the post-bioassay recovery period.

Equations for thermal resistance lines and upper lethal temperatures (LD50) for 5760 min exposure for samples of ribbed mussels, soft-shell clams and blue mussels acclimated at 5 and 15‰ S, and at 5, 15 or 25 C and subjected to bioassay at 15 or 30‰ S are shown in Table 2.

Before further statistical comparisons were made, it was necessary to test the null hypothesis that the variances of the arrays common to each line of regression are equal. Bartlett's test for homogeneity of variances was used to test this hypothesis. The resulting Chi-square value was 2.7102 with 17 degrees of freedom. This value is not significant ($P > .05$) and therefore the null hypothesis is accepted. Further analyses using parametric statistics are now appropriate.

TABLE 1

Summary of the order of polynomial equations which provided the most adequate fit to the data for zero-time post-bioassay period thermal resistance lines for the molluscs *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis*. Numbers in the body of the table refer to the order of the polynomial equation which provided the most adequate description of the data.

Acclimation salinity (‰ S)	Acclimation temperature (C)	Test salinity (‰ S)	Order of polynomial equation		
			Modiolus demissus	*Mya arenaria*	*Mytilus edulis*
15	5	15	2	2	2
	15	15	1	1	2
	25	15	1	1	1
30	5	30	2	2	2
	15	30	1	2	2
	25	30	1	1	1

TABLE 2

Equations for resistance lines, regression coefficients, 95% confidence limits for regression coefficients, LD50, based on 5760-min exposure, 95% confidence limits for these calculated lethal temperatures in samples of *Modiolus demissus*, *Mya arenaria*, and *Mytilus edulis*, acclimated at 15 or 30‰ S and at 5, 15 or 25 C and bioassayed at 15 or 30‰ S.

Species	Acclim. Sal. (‰ S)	Acclim. Temp. (C)	Bioassay Salinity (‰ S)	Equation for resistance line	Regression coefficient with 95% c.l.	LD50 with 95% c.l.
Modiolus demissus	15	5	15	y=45.8154-2.5099x	-2.5099 ±0.4139	36.38 ±0.45
		15	15	y=47.2092-2.8223x	-2.8223 ±0.5544	36.60 ±0.50
		25	15	y=48.0879-2.9326x	-2.9326 ±0.1754	37.06 ±0.20
		5	30	y=44.9877-2.0943x	-2.0943 ±0.7034	37.11 ±0.79
	30	15	30	y=45.7927-2.2879x	-2.2879 ±0.4702	37.19 ±0.47
		25	30	y=47.2254-2.6163x	-2.6163 ±0.3932	37.39 ±0.41
Mya arenaria	15	5	15	y=38.4809-2.7268x	-2.7268 ±0.3200	28.23 ±0.33
		15	15	y=39.3392-2.6110x	-2.6110 ±0.5266	29.52 ±0.69
		25	15	y=39.9719-2.7361x	-2.7361 ±1.1703	29.68 ±1.04
		5	30	y=38.5656-2.8908x	-2.8908 ±0.3210	27.70 ±0.32
	30	15	30	y=39.4247-2.7921x	-2.7921 ±0.1864	28.93 ±0.23
		25	30	y=40.8491-2.8271x	-2.8271 ±0.6853	30.22 ±0.51
Mytilus edulis	15	5	15	y=40.4410-3.8342x	-3.8342 ±0.2121	26.03 ±0.19
		15	15	y=42.3963-4.0578x	-4.0578 ±0.3051	27.14 ±0.29
		25	15	y=40.8286-3.7598x	-3.7598 ±0.9063	26.69 ±0.93
		5	30	y=41.0710-4.1087x	-4.1087 ±0.8337	25.62 ±0.37
	30	15	30	y=42.5630-4.0710x	-4.0710 ±0.3315	27.25 ±0.32
		25	30	y=41.6238-3.5162x	-3.5162 ±1.0021	28.40 ±0.76

A thermal resistance line can be described by two parameters, the adjusted mean (upper lethal temperature) and regression coefficient. It is of interest to determine whether all resistance lines show homogeneity of adjusted means or whether they can be separated into homogeneous sub-groups which can be associated with a variable being considered. The same questions can be posed for the regression coefficients of the thermal resistance lines.

Figure 25 shows the upper lethal temperatures (LD50) as determined for ribbed mussels, soft-shell clams and blue mussels in relation to thermal acclimations of 5, 15 and 25 C, osmotic acclimations of 15 and 30‰ S, and test salinities of 15 and 30‰ S.

The separate effects of species, acclimation temperature and acclimation salinity were determined by factorial analysis of variance (Table 3). The analysis of variance for upper lethal temperatures indicates that significant effects were produced by species and by acclimation temperature.

An *a posteriori* test for multiple comparisons among upper lethal temperatures was performed using the Student-Newman-Kuels test (Sokal and Rohlf, 1969). The results of the multiple comparisons indicate that all upper lethal temperatures, except those listed in Table 4, are significantly different at the 0.05% probability level.

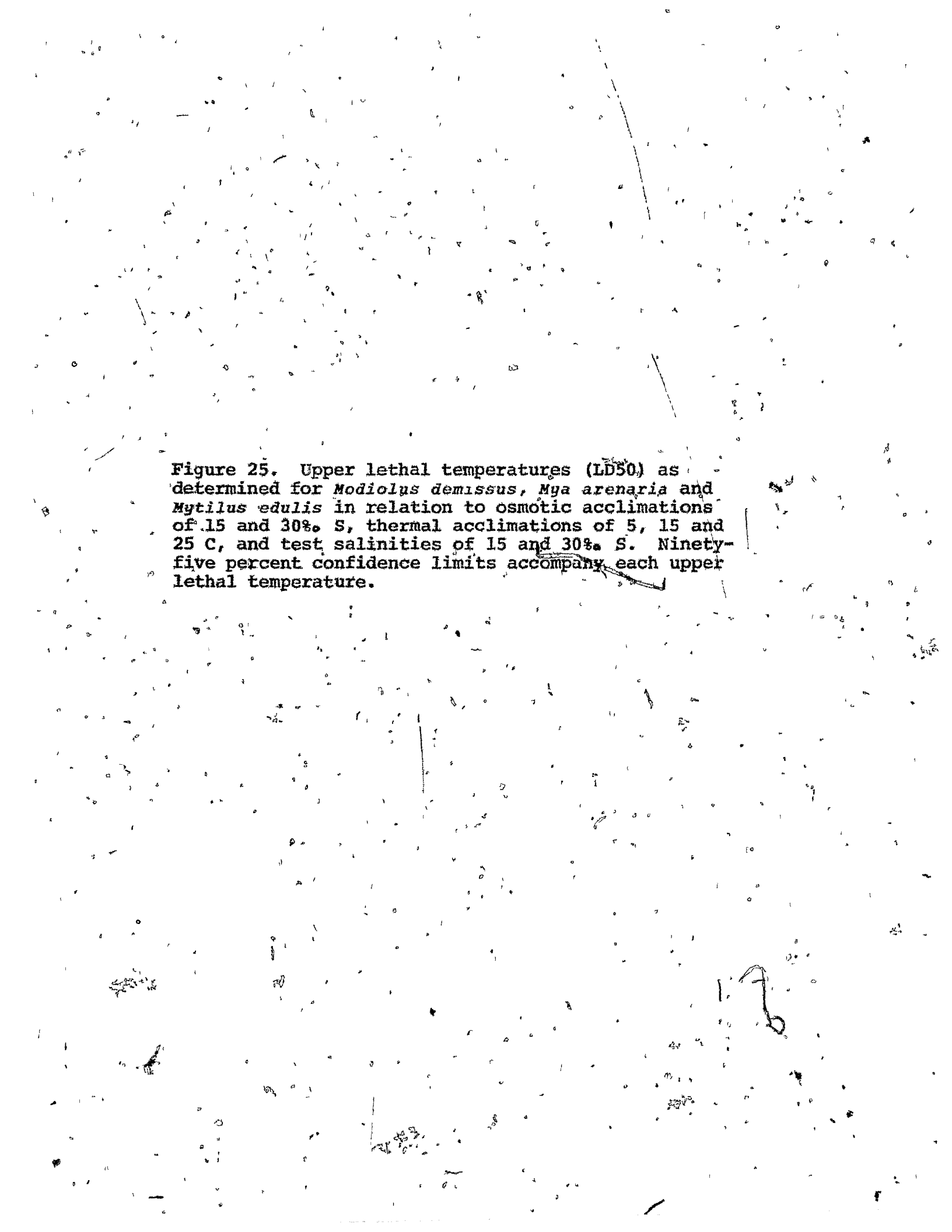


Figure 25. Upper lethal temperatures (LD50) as determined for *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis* in relation to osmotic acclimations of 15 and 30‰ S, thermal acclimations of 5, 15 and 25 C, and test salinities of 15 and 30‰ S. Ninety-five percent confidence limits accompany each upper lethal temperature.

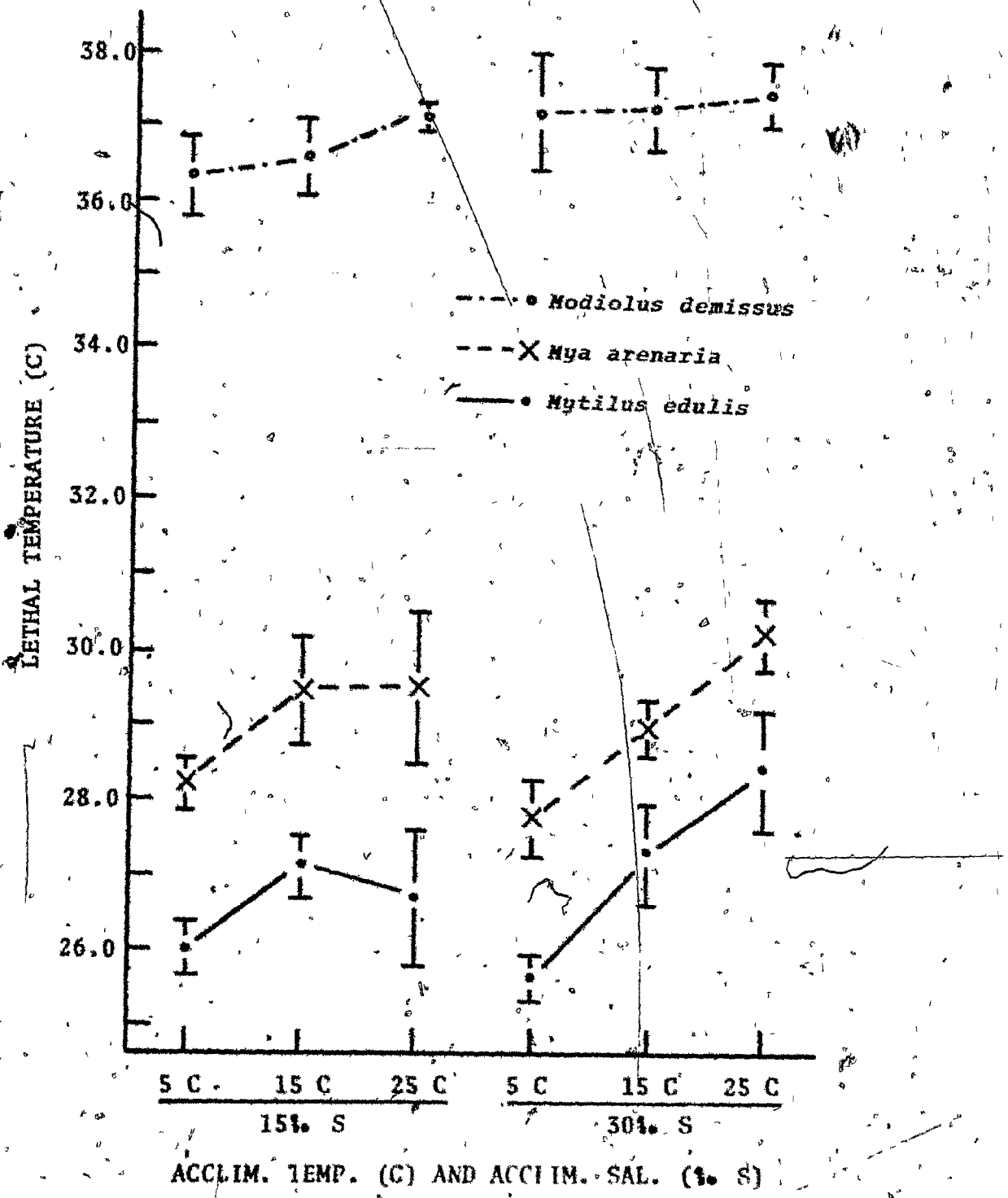

38.0
36.0
34.0
32.0
30.0
28.0
26.0
LETHAL TEMPERATURE (C)
Modiolus demissus
Mya arenaria
Mytilus edulis
5 C
15 C
25 C
5 C
15 C
25 C
15‰ S
30‰ S
ACCLIM. TEMP. (C) AND ACCLIM. SAL. (‰ S)

TABLE 3

Summary of factorial analysis of variance of upper lethal temperatures for samples of *Modiolus demissus*, *Mya arenaria*, and *Mytilus edulis*, for six combinations of thermal and osmotic acclimation.

Treatment	Sum of squares	d.f.	Mean square	Variance ratio	Probability
Species	338.7922	2	169.3961	766.6428	p=0.00001**
Acclimation temp.	6.0636	2	3.0318	13.7212	p=0.0161**
Acclimation salinity	0.3445	1	0.3445	1.5589	p=0.2799
Species/accl. temp.	1.5390	4	0.3848	1.7413	p=0.3021
Species/accl. sal.	0.5014	2	0.2507	1.1347	p=0.4071
Accl. temp./accl. sal.	0.7736	2	0.3868	1.7506	p=0.2844
Error	0.8838	4	0.2210		
Total sum of squares	348.8981				

** highly significant

TABLE 4

Summary of those comparisons among upper lethal temperatures which were not significantly different as determined by the Student-Newman-Kuels test. Samples in each series were tested at a salinity equal to the acclimation salinity level for that series. The table is read row by row. The remaining possible comparisons among upper lethal temperatures are all significantly different.

Species	Acclim. sal. (‰ S)	Acclim. temp. (C)	Up. leth. temp. (C)	Species	Acclim. sal. (‰ S)	Acclim. temp. (C)	Up. leth. temp. (C)
M. demissus	15	5	36.38	*M. demissus*	15	15	36.60
M. demissus	15	25	37.06	*M. demissus*	30	5	37.11
M. demissus	15	25	37.06	*M. demissus*	30	15	37.19
M. demissus	30	5	37.11	*M. demissus*	30	15	37.19
M. demissus	30	15	37.19	*M. demissus*	30	25	37.39
M. demissus	30	5	37.11	*M. demissus*	30	25	37.39
M. arenaria	15	5	28.23	*M. edulis*	30	25	28.40
M. arenaria	15	15	29.52	*M. arenaria*	15	25	29.68
M. edulis	15	15	27.14	*M. edulis*	30	15	27.25

The upper lethal temperatures for ribbed mussels (Table 2) ranged from 36.38 C, for samples acclimated to 5C and 15‰ S, and tested at 15‰ S, to 37.39 C for samples acclimated to 25 C and 30‰ S and tested at 30‰ S. Upper lethal temperatures for soft-shell clams (Table 2) ranged from a low of 27.70 C, for samples acclimated to 5 C and 30‰ S and tested at 30‰ S, and a high of 30.22 for samples acclimated to 25 C and 30‰ S and tested at 30‰ S. The lowest upper lethal temperature obtained for blue mussels (Table 2) was 25.62 C for acclimation to 5 C and 30‰ S and tested at 30‰ S. The highest upper lethal temperature was 28.40 C for samples acclimated to 25 C and 30‰ S and tested at 30‰ S.

Figure 26 shows the relation between the regression coefficients of individual thermal resistance lines, and the acclimation conditions from which they were determined, for each species. Table 5 presents the results of the factorial analysis of variance which was performed on the regression coefficients.

Analysis of variance among regression coefficients indicates that significant effects were produced by species. The first order interactions for species/acclimation temperatures and for species/acclimation salinities are also significant but these require cautious evaluation since the experimental design did not provide replicates "within cells"

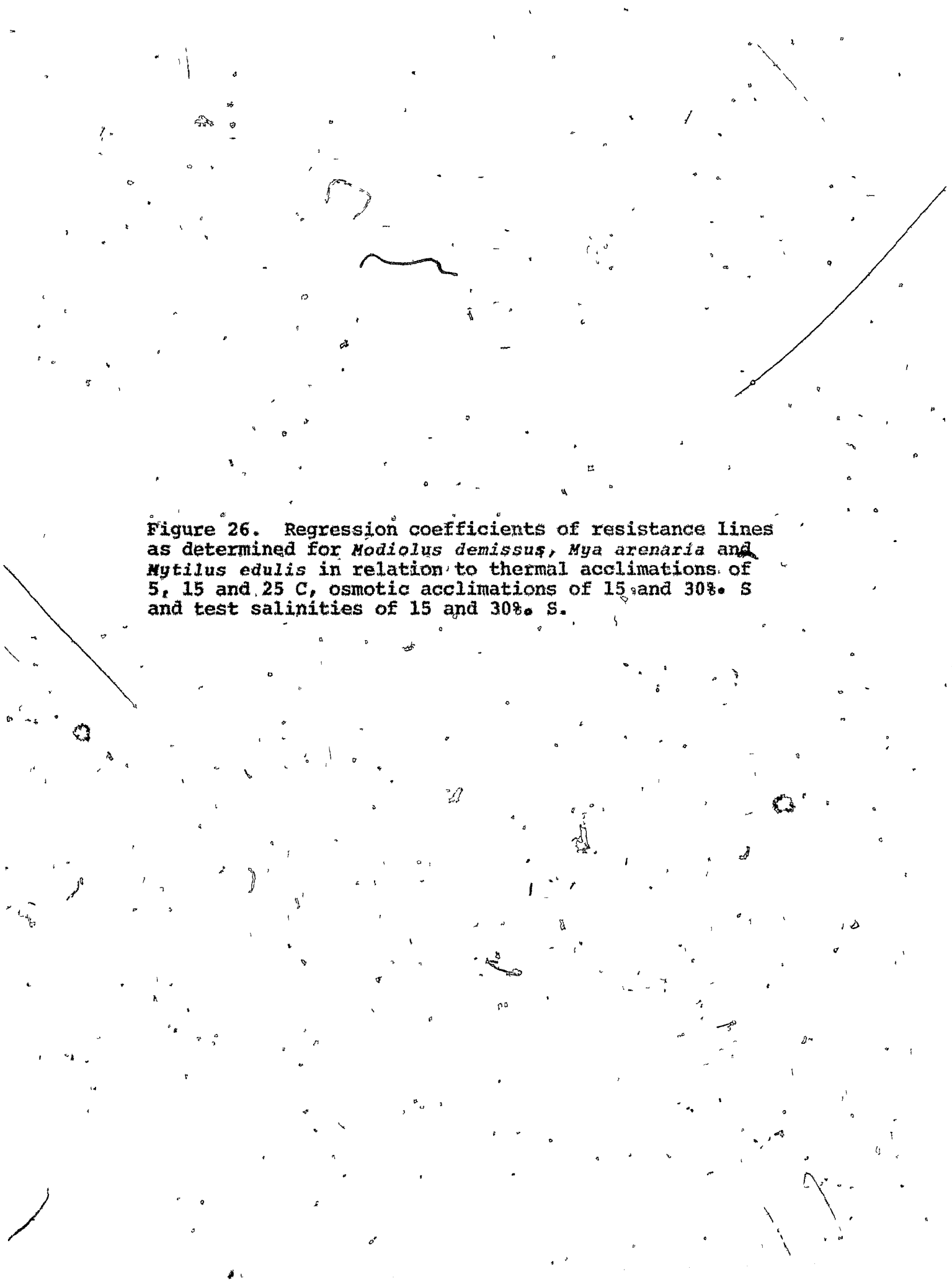


Figure 26. Regression coefficients of resistance lines as determined for *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis* in relation to thermal acclimations of 5, 15 and 25 C, osmotic acclimations of 15 and 30‰ S and test salinities of 15 and 30‰ S.

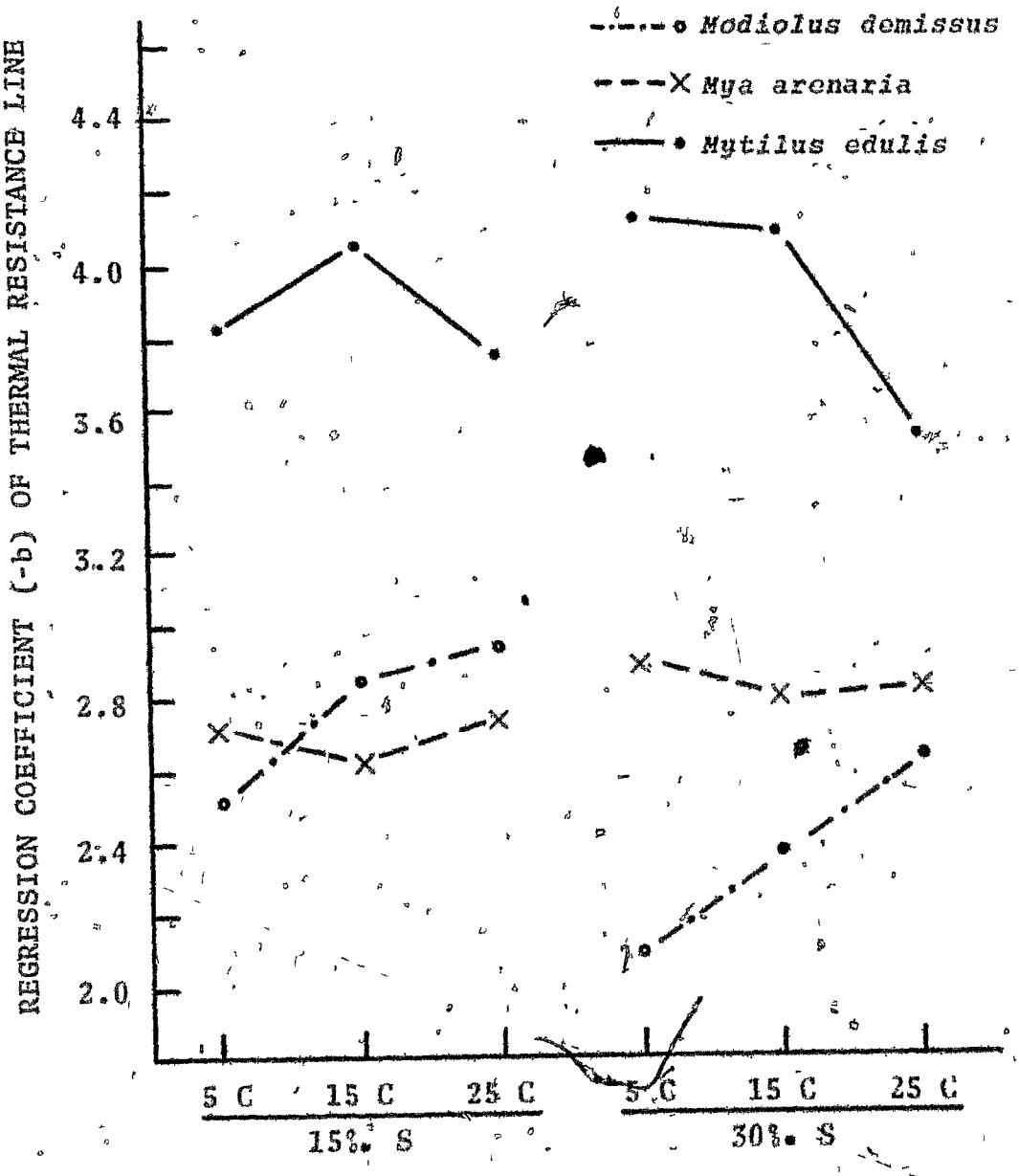

Modiolus demissus
Mya arenaria
Mytilus edulis
REGRESSION COEFFICIENT (-b) OF THERMAL RESISTANCE LINE
4.4
4.0
3.6
3.2
2.8
2.4
2.0
5 C
15 C
25 C
15‰ S
5 C
15 C
25 C
30‰ S
ACCLIM. TEMP. (C) AND ACCLIM. SAL. (‰ S)

TABLE 5

Summary of factorial analysis of variance of regression coefficients of resistance lines for samples of *Modiolus demissus*, *Mya arenaria*, and *Mytilus edulis*, from six combinations of thermal and osmotic acclimation.

Treatment	Sum of squares	d.f.	Mean square	Variance Ratio	Probability
Species	6.2695	2	3.1346	210.2337	p=.00009 **
Acclimation temp.	0.0190	2	0.0095	0.6378	p=.57490
Acclimation salinity	0.0343	1	0.0343	2.3024	p=.2038
Species/accl. temp.	0.4180	4	0.1045	7.0084	p=.0429 *
Species/accl. sal.	0.2649	2	0.1324	8.8843	p=.0338 *
Accl. temp./accl. sal.	0.0217	2	0.0108	0.7270	p=.5379
Error	0.0596	4	0.0149		
Total sum of squares	7.0870				

* significant
** highly significant

which provides the source of "within-cell" variance for the interactions.

The results of the test for homogeneity of regression coefficients (Li, 1964) indicate that heterogeneity exists among the 18 regression coefficients that were tested (Table 6). This is indicated by the fact that the variation among b-components is significant ($P < 0.001$). This leads to the rejection of the null hypothesis which stated that the regression coefficients of the 18 regression lines are equal. The rejection of the null hypothesis necessitates the application of an *a posteriori* test for differences among a set of regression coefficients. The method used in this case is the Simultaneous Test Procedure (STP) as described by Sokal and Rohlf (1969).

Table 7 presents a summary of the Simultaneous Test Procedure. Homogeneity of regression coefficients occurs within each species as well as between ribbed mussels and soft-shell clams. However, heterogeneity of regression coefficients is indicated between blue mussels and soft-shell clams as well as between blue mussels and ribbed mussels.

Figures 27 to 29 show the thermal resistance lines for 10% and 50% mortality for ribbed mussels, soft-shell clams and blue mussels when acclimated to 25 C and 30‰ S. Parameters of the thermal resistance lines are shown in

TABLE 6

Summary for test of homogeneity of regression coefficients of resistance lines for samples of *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis*, from six combinations of thermal and osmotic acclimation.

Source of Variation	Sum of squares	d.f.	Mean square	F	Probability
Regression due to b	254.8710	1	254.8710	2831.9000	p<0.001**
Variation among b components	19.6090	17	1.1535	12.8167	p<0.001**
Pooled residual	7.6500	85	0.0900		
Within sample	482.8618	103			

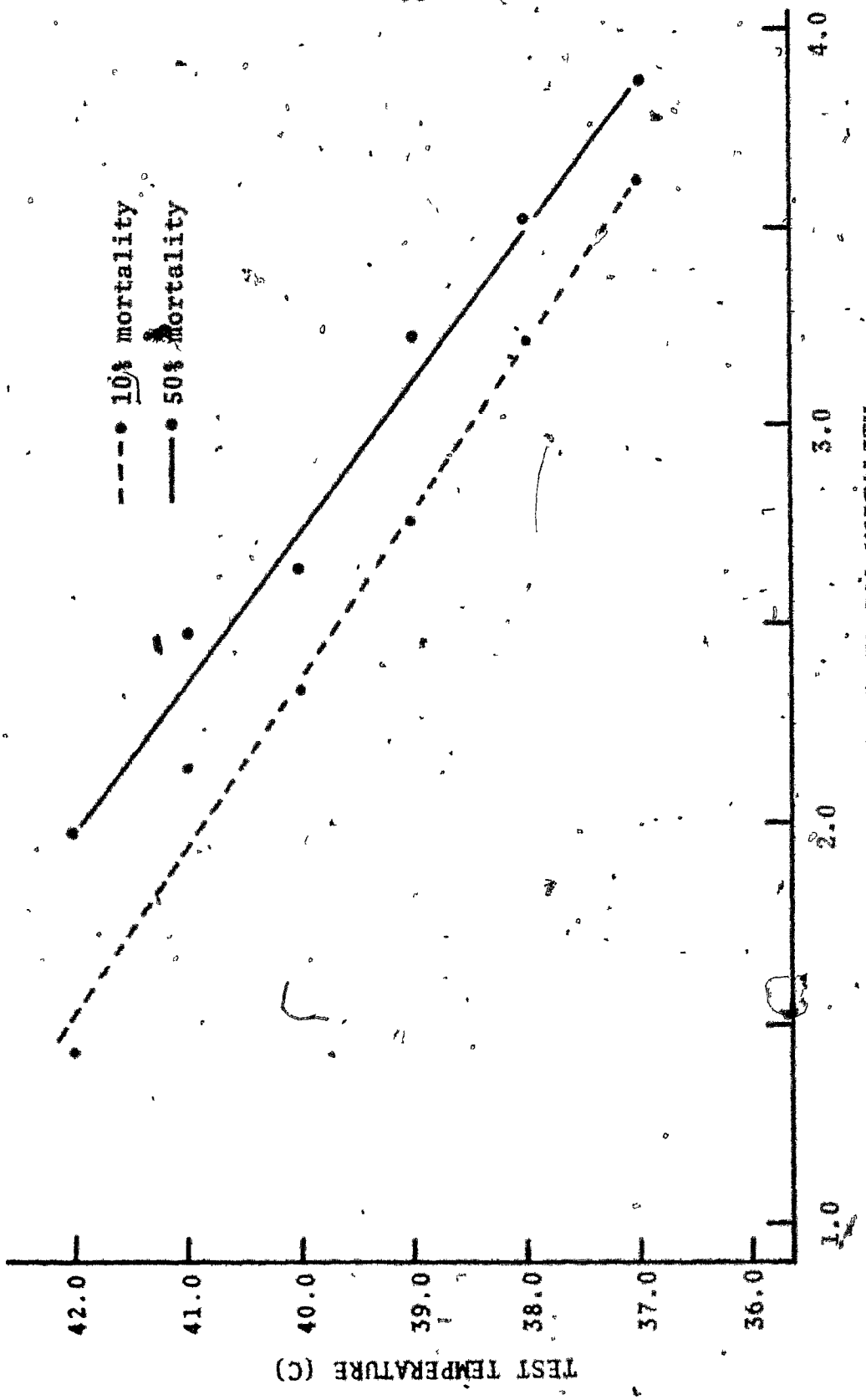
** highly significant

TABLE 7

Summary of the Simultaneous Test Procedure for differences among the regression coefficients of the thermal resistance lines for samples of *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis*, from six combinations of thermal and osmotic acclimation. Ninety-five per cent confidence limits accompany each regression coefficient. Homogeneity of regression coefficients is indicated by brackets which connect treatments.

Species	Treatments: Acclim. Sal. (‰ S)	Acclim. temp. (°C)	Test sal. (‰ S)	Regression coefficient	95% c.l.	Homoegeneity of reg. coef.
Modiolus demissus	15	5	15	-2.5099	±0.41	
		15	15	-2.8223	±0.55	
		25	15	-2.9326	±0.18	
	30	5	30	-2.0943	±0.70	
		15	30	-2.2879	±0.47	
		25	30	-2.6163	±0.39	
Mya arenaria	15	5	15	-2.7268	±0.32	
		15	15	-2.6110	±0.53	
		25	15	-2.7361	±1.17	
	30	5	30	-2.8908	±0.32	
		15	30	-2.7921	±0.19	
		25	30	-2.8271	±0.69	
Mytilus edulis	15	5	15	-3.8342	±0.21	
		15	15	-4.0578	±0.30	
		25	15	-3.7598	±0.91	
	30	5	30	-4.1087	±0.83	
		15	30	-4.0710	±0.33	
		25	30	-3.5162	±1.00	

Figure 27. Thermal resistance lines for 10% and 50% mortality response levels for 28th day of post-bioassay recovery period for samples of *Modiolus demissus* acclimated to 30‰ S, 25 C and tested at 30‰ S.


10% mortality
50% Mortality
TEST TEMPERATURE (C)
42.0
41.0
40.0
39.0
38.0
37.0
36.0
LOG TIME (MIN) TO 50% MORTALITY
1.0
2.0
3.0
4.0

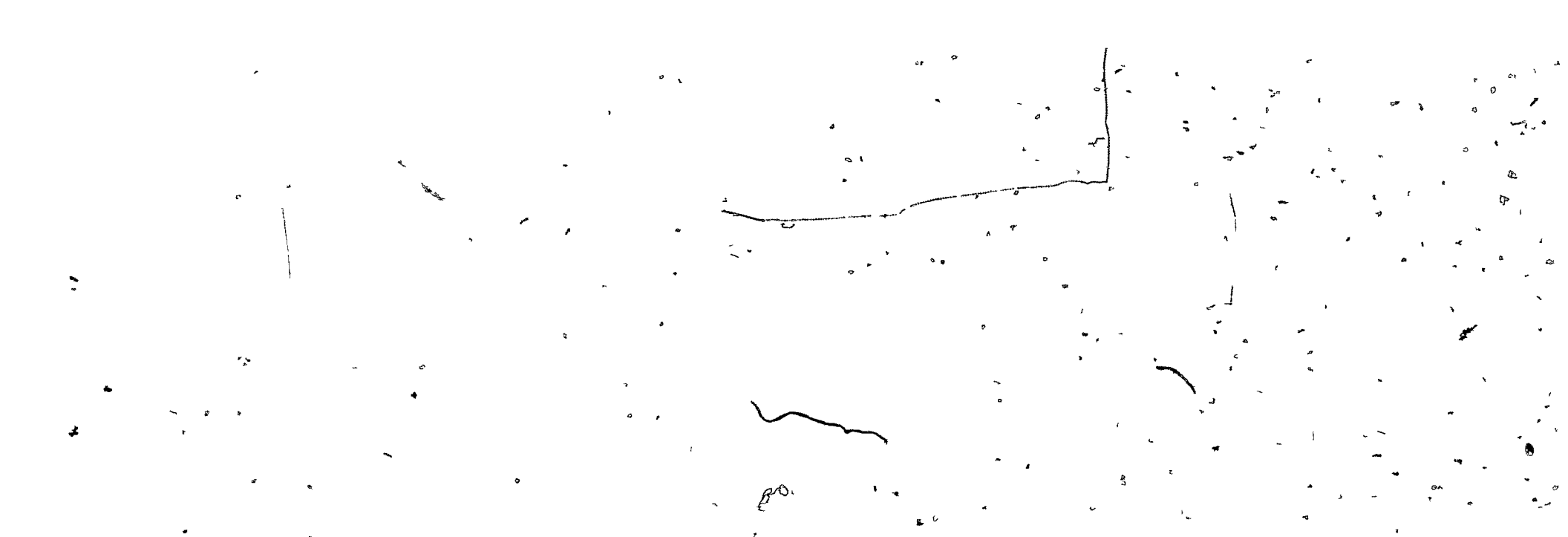


Figure 28. Thermal resistance lines for 10% and 50% mortality response levels for 28th day of post-bioassay recovery period for samples of *Mya arenaria* acclimated to 30‰ S, 25 C and tested at 30‰ S.

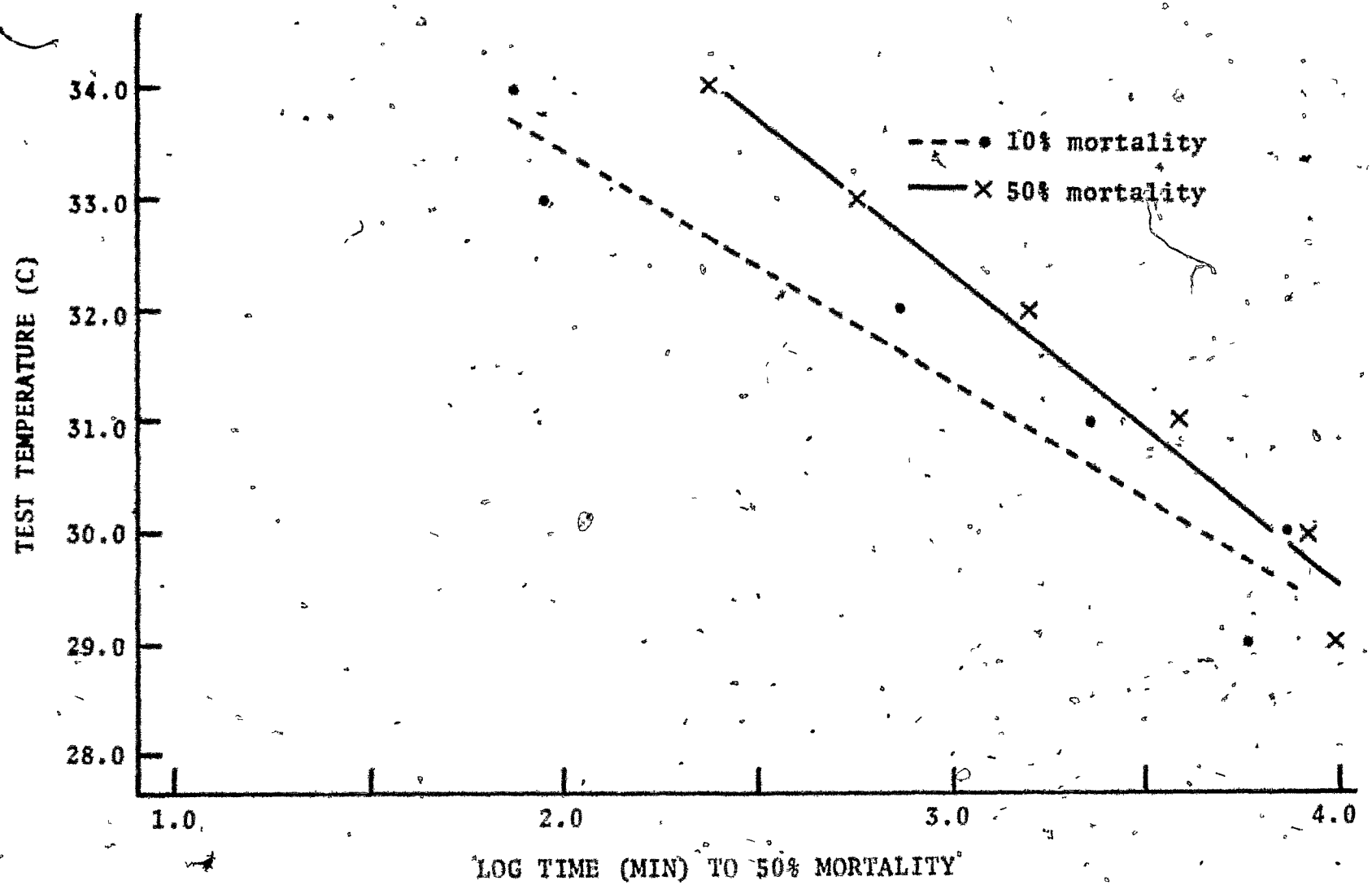

34.0
33.0
32.0
31.0
30.0
29.0
28.0
TEST TEMPERATURE (C)
10% mortality
50% mortality
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY

Figure 29. Thermal resistance lines for 10% and 50% mortality response levels for 28th day of post-bioassay recovery period for samples of *Mytilus edulis* acclimated to 30‰ S, 25 C and tested at 30‰ S.

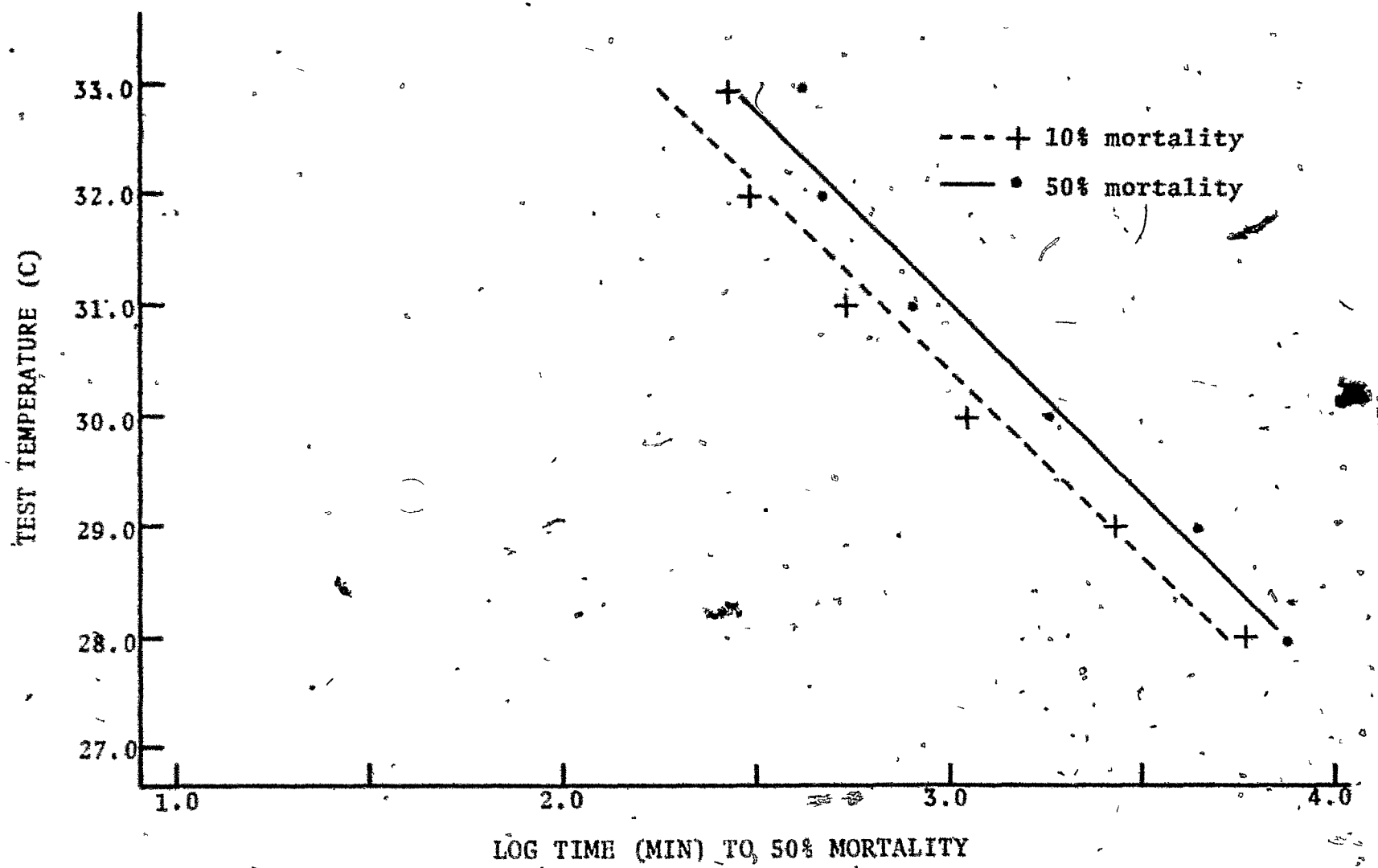

33.0
32.0
31.0
30.0
29.0
28.0
27.0
TEST TEMPERATURE (C)
1.0
2.0
3.0
4.0
LOG TIME (MIN) TO 50% MORTALITY
10% mortality
50% mortality

Table 8. Results of the statistical comparisons are presented in Table 9.

TABLE 8

Summary of parameters of thermal resistance lines for 10% and 50% mortality response level. Equations for thermal resistance lines, regression coefficients and upper lethal temperatures (LD10, LD50) are shown for samples of *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis*, acclimated to 30‰ S, 25 C and bioassayed at 30‰ S.

Species	Acclim. Sal. (‰)	Acclim. temp. (C)	Test Sal. (‰)	Response level (%)	Equation for thermal resistance line	Reg. coef.	Lethal temp. (C)
Modiolus demissus	30	25	30	10	y=45.5844-2.3531x	-2.3531	36.74
				50	y=47.2254-2.6163x	-2.6163	37.39
Mya arenaria	30	25	30	10	y=37.5210-2.0411x	-2.0411	29.85
				50	y=40.8491-2.8271x	-2.8271	30.22
Mytilus edulis	30	25	30	10	y=40.6648-3.4060x	-3.4060	27.86
				50	y=41.6238-3.5162x	-3.5162	28.40

TABLE 9

Summary of Students t-test for comparisons between LD10 and LD50, and between regression coefficients of thermal resistance lines for 10% and 50% mortality response levels, for samples of *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis* acclimated to 30‰ S, 25C and tested at 30‰ S.

Species	LD10	LD50	d.f.	t	P
Modiolus demissus	36.74	37.39	10	3.5951	P<0.01
Mya arenaria	29.85	30.22	10	1.6712	P>0.05
Mytilus edulis	27.86	28.40	10	2.3256	P<0.05

Species	Regression coefficient 10%	Regression coefficient 50%	d.f.	t	P
Modiolus demissus	-2.3531	-2.6163	10	1.2554	P>0.05
Mya arenaria	-2.0411	-2.8271	10	0.9086	P>0.05
Mytilus edulis	-3.4060	-3.5162	10	0.2203	P>0.05

DISCUSSION

Post-bioassay mortality

A technique for determination of the time to 50% mortality in a thermal bioassay is described by Fry, Hart and Walker (1946), Fry (1947). Their approach is based on the classical pharmacological method of bioassay (Finney, 1971). The technique consists of recording the time to death for each animal of a sample in a bioassay. The time to 50% mortality is determined by either a geometric mean or by probit analysis. Both procedures provide virtually the same result.

One of the problems encountered in using the method as described by Fry (1947) is that it is not conducive to the further examination of any survivors of a particular bioassay. In addition, time to 50% mortality is determined from a series of observations on the times to death for single animals. An aberrant response on the part of any test subject carries as much weight as any other in the determination of a time-mortality line for that bioassay.

In the present study, percentage mortality was recorded for subsamples which were removed at arbitrarily determined time intervals. Time to 50% mortality was determined by probit analysis for each subsample in the sample.

The immediate advantage of this method is that it provides a mechanism whereby further analysis can be conducted on any survivors in any set of test conditions. Percentage mortality as determined for each subsample represents a mean percentage mortality for that particular set of test conditions.

The times to 50% mortality during the 28-day post-bioassay recovery period are shown in Figures 7 to 24 (part a). In most cases, the time to 50% mortality decreases rapidly during the first 10 to 12 days of the post-bioassay recovery period. By the 28th day, time to 50% mortality has become relatively stable.

Percentage mortality for post-bioassay control groups was less than 10%. Mortality responses of this level indicate the absence of appreciable acute stress during the post-bioassay recovery period. Therefore, decreases in time to 50% mortality during the post-bioassay recovery period can be attributed, at least in part, to the latent effects of the acute thermal stress which was imposed on the animals during thermal bioassay.

Information pertaining to the extended effects of a terminated thermal stress is scant. The general conclusions of a series of studies concerned with the effects of the severe winter of 1962/1963 on marine life along the British coasts (Crisp, 1964) reveal that the full effects of thermal

stress resulting from exposure to lower temperatures was not manifest until several weeks after the thermal exposure. Dickie and Medcof (1963) report similar circumstances for scallops (*Placopecten magellanicus*) which were exposed to extremely high temperatures in the southern Gulf of St. Lawrence (Canada). Smith (1973) reported that both *Gammarus pseudolimnaeus* and *Gammarus lacustris*, when acclimated to 18 C, had an upper lethal temperature of 26 C when exposed to higher temperatures for 5760 min. However, the upper lethal temperatures were reduced to 24 C and 25 C respectively when the animals were held, following bioassay, for an additional 26 days at 18 C.

Thermal resistance lines were calculated for zero-time post-bioassay recovery and for the 28th day of post-bioassay recovery. These are illustrated in Figures 7 to 24 (part b). When a logarithmic transformation is applied to the times to 50% mortality, the calculated thermal resistance line is usually linear (Fry, Hart and Walker, 1946). In the present work, logarithmic transformation of the times to 50% mortality for the formulation of a thermal resistance line for zero-time post-bioassay recovery does not consistently result in a thermal resistance line which is linear.

Zero-time post-bioassay thermal resistance lines are curvilinear for all species when samples are thermally acclimated to 5C, regardless of the level of osmotic acclimation (Table 1). Second order polynomial equations were

used to describe these thermal resistance lines since they provided a more adequate description of the data than did the first order polynomial equations. Linear thermal resistance lines result for all species when samples are acclimated to 25 C regardless of the level of osmotic acclimation. When samples are acclimated to 15 C both linear and curvilinear thermal resistance lines result (Table 1). In this case, no apparent relation exists between treatment and the order of the polynomial equation which most adequately describes the data.

A strong correlation exists if the temperature increment between the level of thermal acclimation and the temperatures at which samples were subjected to bioassay is correlated with the order of the polynomial equation which is used to describe the data. The smaller the temperature increment, the greater is the probability that the thermal resistance line will be linear.

A plausible explanation for the existence of curvilinear thermal resistance lines is a probable lag in thermal equilibration time which would act as a buffer between the animal and the stimulus as it is transferred from acclimation to bioassay conditions. This interval would be an inverse function of the temperature change. Thus, the thermal equilibration time would represent increasingly

larger proportions of the time interval required to elicit a specific response as the bioassay temperature is increased.

Consideration of any of the curvilinear thermal resistance lines will show that the portion of the line associated with low test temperatures is essentially linear whereas the portion of the line associated with the higher test temperatures is markedly skewed to the right (Fig. 7(b). At low test temperatures, the thermal equilibration time is probably small in relation to the time interval required to elicit a 50% mortality response. As the test temperature is increased, the lag for thermal equilibration would represent increasingly larger proportions of the time to 50% mortality. Thus, the resistance line would exhibit a progressive increase in deviations from linearity with increasing levels of test temperature.

Suspected causes of death of animals at higher temperatures are many but poorly demonstrated. Kinne (1970) states that "evidence from field observations suggests that the primary cause of thermal death at higher temperatures is related to the breakdown in physiological integration rather than to direct cell damages". Heat of chill coma usually precedes actual cell damage and critically incapacitates the individual concerned. Brett (1956) suggests

that the thermal lethal level must be set by the resistance of the most sensitive system. For fish, this has been indicated to be the nervous system (Brett, 1956). However, Hielbrunn *et al.* (1946) state that muscle tissue of fish is more readily inactivated by high temperatures than is tissue of the nervous system. Higher temperatures tend to cause an insufficient supply of oxygen (Battle, 1926); enzymes pass the phase where inactiviation exceeds activation and synthesis (Somero, 1969); and there may be alteration of the lipid bilayer of cellular membranes (Chapman, 1967). At still higher temperatures protein denaturation may occur (Prosser, 1958) and toxic substances may be released from cells (Kinne, 1963). Brett (1952) shows that non-linear thermal resistance lines result for young coho, chum and sockeye salmon acclimated to high temperatures and subjected to lower lethal temperatures. The suggestion is made that thermal death at temperatures approaching 0 C results from more than one cause. Therefore, it is not without precedence that curvilinear thermal resistance lines could result from thermal bioassays which encompass a relatively broad temperature range.

Upper lethal temperatures

The results of the analysis of variance on upper lethal temperatures indicate that acclimation temperature and species components are highly significant. The acclimation salinity component was not significant.

The underlying relationships for 'within species' upper lethal temperatures were examined by the Student-Newman-Kuels test. In general, increases in upper lethal temperatures for these comparisons are positively correlated with increases in the level of thermal acclimation. A 'species effect' on upper lethal temperatures was statistically tested by the same method. The results indicate that levels of upper lethal temperatures show a high degree of species-specificity.

Upper lethal temperatures within species

Upper lethal temperatures of *Modiolus demissus*

The highest upper lethal temperature for ribbed mussel was 37.39 C for samples acclimated to 25 C and 30‰ S. Since this upper lethal temperature was determined for a specific exposure period, it cannot be considered as an incipient or an ultimate upper lethal temperature. This statement applies to all upper lethal temperatures reported in this study unless otherwise indicated. Incipient and ultimate upper lethal temperatures are measures of tolerance

and are determined from thermal exposure of animals which are of sufficient time duration to ensure that death of more than a specific proportion of the sample, does not result from thermal conditions.

Waugh and Garside (1971) reported that the highest upper lethal temperature determined for this species was 40.18 C for a 1440 min exposure period for thermal acclimation of 25 C and osmotic acclimation of 25‰ S. Recalculation of the present data for a similar time exposure gives an upper lethal temperature of 38.96 C. Waugh (1972) reported an upper lethal temperature of 39.46 C for ribbed mussels exposed to elevated temperatures for 1440 min. Mean monthly temperature of the substrate at the collection site was 24.2 C at the time of sampling. Read and Cumming (1967) reported a value of 40 C which was obtained by placing the animals in a test bath at approximately 20 C and subsequently increasing the temperature one degree each 3.5 days. The upper lethal temperature recorded was that temperature at which the last animal died in the test sample. Vernberg *et al.* (1963) reported an upper lethal temperature of 43.5 C, based on 150-min exposure of gill tissue from samples of ribbed mussels acclimated to 25‰. An upper lethal temperature of 41.7 C was obtained from the present data for a similar time exposure for samples of whole animals acclimated to 25 C and 30‰ S. Lent (1968) states that the upper lethal temperature for ribbed mussel is 36.4 C for animals mechanically

closed and 37.8 for air-gaping animals when subjected to a 10 hr thermal exposure in air at 80% humidity. Consideration of the above data indicates that the present results are quite similar to those obtained by several other methods.

Upper lethal temperatures for thermal acclimations of 5, 15 and 25 C in a medium of 30‰ S are not significantly different from each other. However, upper lethal temperatures for similar levels of thermal acclimation in a medium of 15‰ S are significantly different. They increase with increasing levels of thermal acclimation.

Upper lethal temperatures for corresponding levels of thermal acclimation are significantly lower for osmotic acclimation to 15‰ S than for osmotic acclimation to 30‰ S. A similar trend in upper lethal temperatures for ribbed mussels was shown by Waugh and Garside (1971). They reported that upper lethal temperatures increase as the level of osmotic acclimation is increased provided that the salinity of the test medium is similar to that of the osmotic acclimation medium. They reported this trend for all levels of thermal acclimation.

Upper lethal temperatures of *Mya arenaria*

Upper lethal temperatures for soft-shell clams increase with increasing levels of thermal acclimation for 30‰ salinity acclimation. The lowest upper lethal temperature was 27.70 C for acclimation conditions of 5 C and

30‰ S. The highest upper lethal temperature was 30.22 C for a thermal acclimation of 25 C and an osmotic acclimation of 30‰ S. A similar pattern emerges for osmotic acclimations at 15‰ S except that the upper lethal temperature for a thermal acclimation of 25 C is not significantly different from the corresponding value for a thermal acclimation at 15 C. The different levels of osmotic acclimation exert only a minor effect on the upper lethal temperatures for comparable levels of thermal acclimation. In contrast, Theede and Lassig (1967) present average survival times for isolated gill tissue of *Mya arenaria* which was acclimated to various salinity levels. The average survival times were 26, 50 and 178 min for cilia tested at 36 C and at salinity levels of 6, 15 and 30‰. Their results indicate a high degree of interaction among average survival times and acclimation salinity levels.

Kennedy and Mihursky (1971) report upper lethal temperatures for the soft-shell clam. Their determinations were made on three size ranges: newly set (1.5-4 mm), young-of-the-year (14-23 mm) and adults (45-76 mm). Samples were tested for 1440 min in a medium that fluctuated erratically from 11.2 to 17‰ S. Upper lethal temperatures of the adult samples when acclimated to 5, 15 and 25 C were 30.3, 30.5 and 32.1 C respectively. The present data for thermal acclimations at 5, 15 and 25 C for 15‰ S osmotic acclimation were recalculated to estimate upper lethal

temperatures for 1440 min exposures. The resulting upper lethal temperatures were 29.87, 31.09, and 31.33 C, respectively. Henderson (1929) reported an upper lethal temperature of 40.6 C for samples of soft-shell clams held in full sea water which was heated at the rate of one degree every 5 min. Henderson's result is not comparable with those of the present study because of the difference in procedure.

Upper lethal temperatures of *Mytilus edulis*

The highest upper lethal temperature obtained for blue mussels was 28.40 C. This value was obtained for samples acclimated to 25 C and 30‰ S. Read and Cumming (1967) report an upper lethal temperature of 30 C for the blue mussel when the temperature of the test bath was increased at the rate of one degree every 3.5 days. Henderson (1929) obtained an upper lethal temperature of 40.8 C for samples tested in full sea water which was heated at the rate of one degree every five minutes.

Upper lethal temperatures increase slightly, but significantly with increasing levels of thermal acclimation when the level of osmotic acclimation is held at 30‰ S. Osmotic acclimations to 15‰ S result in upper lethal temperatures which increase over the lower levels of thermal acclimations but decrease at the upper level. This pattern is very similar to that observed in the upper lethal temperatures for the soft-shell clam.

The level of osmotic acclimation exerts only a small effect on the upper lethal temperatures obtained from similar levels of thermal acclimation. Schlieper and Kowalski (1956) report that survival time of gill tissue of blue mussel is lower at 15‰ S than at 30‰ S when tests were conducted at 35 C. This was confirmed by Theede and Lassig (1967). They obtained samples of blue mussel populations which were located at Büsum on the North Sea, Kiél and Tuarmine on the Baltic. Salinity at these sites approximated 30, 15 and 6‰ respectively. Prior to experiments, the samples were kept at a constant temperature of 10 C and at their respective environmental salinity. Tests were conducted on isolated gill tissue . The results show that the average survival time at 36 C was 82 min at 30 S, 37 min at 15‰ and 21 min at 6‰ S.

Upper lethal temperatures among species

Results of the Student-Newman-Kuels test for multiple comparisons among means indicate that the upper lethal temperatures determined for ribbed mussel are significantly higher than those determined for both the soft-shell clam and blue mussel. Upper lethal temperatures for soft-shell clams are significantly higher than those determined for the blue mussel at similar acclimation conditions. However, Figure 25 indicates that there is a small degree of overlap in upper lethal temperatures for

soft-shell clams and blue mussels when upper lethal temperatures of these two species are compared for different acclimation conditions. Thus it would appear that upper lethal temperatures are species-specific.

Species-specificity for thermal response has been shown to exist at the molecular and cellular level for molluscs. Read (1963) determined thermal inactivation points for preparation of aspartic/glutamic transaminase obtained from three species of mytilids which inhabit different positions in the subtidal and intertidal zones. Preparations were assayed at 56 C for 12 min. Enzymes from the subtidal mollusc *Modiolus modiolus* yielded the highest degree of thermal inactivation and preparations from the high intertidal mollusc *Brachidontes demissus* exhibited only minor thermal inactivation. Preparations from the low intertidal mollusc *Mytilus edulis* showed an intermediate degree of thermal inactivation.

Zhirmunsky (1967) reported that marine invertebrates are characterized by a certain species-specificity in relation to cell thermostability. The species-specificity of thermostability was established by determining the survival time of ciliated epithelial cells when tested in a range of temperatures. The temperature causing thermonarcosis of ciliated epithelial cells in one min was used to compare levels of cell thermostability within and among species. In this case, the temperature causing thermonarcosis in one min is

analogous to an upper lethal temperature which causes a specific mortality response within a defined exposure period. Cell thermostability levels were obtained for 14 species which included echinoderms, molluscs and coelenterates. The results indicated a considerable conservation of cell thermostability within each species. The temperature level of cell thermostability in different populations gathered either in different or in the same waters, but at various depths, was virtually identical. The study of Theede (1972) can be used to show that widely separated populations of *Mytilus edulis* (North Sea and Cape Cod) exhibit only slight differences in their cellular thermal resistance and cellular upper lethal temperatures.

Dzhamusova (1967) obtained muscle thermostability lines for seven species of littorinids by measuring the time to irreversible loss of muscle excitability in response to an induction current at a series of test temperatures. The seven species are characterized by different muscle thermostability temperatures for a specified exposure time. Dzhamusova states that

> differences in muscle thermostability of closely related species give every reason to consider muscle heat-resistance as a cytophysiological species criterion.

Reshöft (1961) reports species-specific differences in cellular thermal resistance among the three lamelli-

branch species, *Spisula solida*, *Modiolus modiolus* and *Mytilus edulis*. The three species occur at different depths in the North Sea. *Spisula* occurs at the greatest water depth and exhibits thermal resistance at temperatures well below those at which thermal resistance begins in *Mytilus* which inhabits shallower waters. Schlieper (1966) has shown that cilia of isolated gill pieces of the subtidal mollusc *Aequipecten irradians* ceased beating when exposed to 37 C for 100 min whereas Vernberg *et al.* (1963) indicated that isolated gill pieces of the intertidal molluscs *Modiolus demissus* and *Crassostrea virginica* survived 44 C for the same period of time. Schlieper (1966), Schlieper *et al.* (1967) exposed isolated gill pieces of marine molluscs to a temperature increase of one degree per 5 min interval. Gill tissue of the tropical species *Chama cornucopia* showed thermal tolerance over a range of temperatures which were lethal to gill tissue of the temperate species *Mytilus edulis*.

Data presented by Zhirmunsky (1967) indicate that there is a high degree of correlation between the species-specific temperature for cell thermostability of intertidal molluscs and the latitudinal distribution of the species. Tropical species tend to exhibit higher thermal levels for cell thermostability than do arctic species. However, Zhirmunsky (1960) has shown that the temperate intertidal pelecypod *Modiolus atrata* has a higher thermal level for

cell thermostability than the tropical subtidal pelecypod *Modiolus philippinarum*. Such discrepancies in the relationship among cell thermostabilities and latitudinal distribution of species are in accordance with zoogeographical evidence suggesting in some cases the predominance of vertical zonation (habitat) over latitudinal zonation (range) (Shelford, 1911).

For intact organisms, Sprague (1963) has shown that the "ultimate 24-hr LC50" is species-specific among four species of gammarids (Crustacea). Sprague states that the "*ultimate 24-hr LC 50* ... is comparable to the *ultimate upper incipient lethal temperature* of Fry (1947)".

The results of the present study indicate that upper lethal temperatures of the intact organism are species-specific. In addition, increases in species-specific upper lethal temperatures are positively correlated with specific vertical position of habitat within the intertidal zone.

Thermal resistance

Thermal resistance lines within species

Results of the test for homogeneity of regression coefficients and of the Simultaneous Test Procedure for differences among a set of regression coefficients indicate that the regression coefficients of the thermal resistance

lines for 'within species' comparisons are equal.

Dzhamusova (1967) presents thermostability curves for muscle tissue of molluscs of different populations of *Littorina squalida*. One population was collected from the Sea of Japan which has a summer water temperature of 20 to 23 C. The second population was collected from the Sea of Okhotsk which has a summer water temperature of 10-12 C. The muscle thermostability of the two populations of *Littorina squalida* is characterized by the same straight line. An analogous situation resulted when muscle thermostability lines were determined for populations of *Littorina mandchurica* which were also collected in the Sea of Japan and in the Sea of Okhotsk. This information indicates that separate populations of a species have similar muscle thermostability lines even when there is a considerable difference in habitat temperature of the separate populations.

Thermal resistance lines among species

Homogeneous regression coefficients of thermal resistance lines can be further grouped into two distinct categories. Regression coefficients of the thermal resistance lines for the ribbed mussel and for the soft-shell clam are homogeneous. The mean value of the combined regression coefficients of the thermal resistance lines for ribbed mussel and soft-shell clam is -2.6539 and the range extends from -2.0943 to -2.9326. The regression coefficients of

the thermal resistance lines for blue mussels are not homogeneous when compared to those for either the soft-shell clam or ribbed mussel. The mean of the regression coefficients for the blue mussel is -3.5162 and the range is from -4.1087 to -3.8913. The absolute value of each regression coefficient is used to compare magnitudes of regression coefficients among regression lines which have negative regression coefficients. The result is that the absolute values of the regression coefficients of the thermal resistance lines obtained for blue mussels are larger than the absolute value of the regression coefficients of the thermal resistance lines obtained for either the soft-shell clam or the ribbed mussel.

The work of Dzhamusova (1967) on littorinids indicates that slopes of muscle thermostability lines are a species-specific characteristic. The regression coefficient of the muscle thermostability line for *Littorina squalida* has a smaller absolute value than does that obtained for *Littorina mandchurica*. Zhirmunsky (1967) obtained thermostability lines for ciliated epithelial cells which were isolated from 29 species of intertidal molluscs. These results confirm the work of Dzhamusova (1967) which suggests that the regression coefficients of thermal response lines are specific-specific.

Upper lethal temperatures and regression coefficients of thermal resistance lines

In the general case, the adjusted mean of 50% mortality response line indicates a dose intensity of a defined stimulus at which a 50% mortality response is manifest. The regression coefficient of a 50% mortality response line can be used to describe a range of the dose over which a 50% mortality response is detectable. For a thermal resistance line, the adjusted mean is an upper lethal temperature (LD50) for a standardized time exposure of a sample of animals which has been kept in a defined set of environmental conditions. The regression coefficient of a thermal resistance line is used to describe a range of temperatures, above the upper lethal temperature, over which a 50% mortality response can be detected. This range is the *thermal resistance range*.

Since the regression coefficient of the thermal resistance line can be used to describe the thermal resistance range, it is not necessary to state the actual range unless absolute comparisons among response lines are deemed necessary. Standardized upper and lower limits can be applied to the range of dose intensities of the defined stimulus in order that absolute comparisons can be made among stimulus ranges applied during different experimental conditions. A regression coefficient of a thermal resistance line which is of larger absolute value indicates a broad

thermal resistance range whereas a regression coefficient of smaller absolute value implies a narrow thermal resistance range.

Numerous studies have been conducted to determine thermal responses for molluscs. However, little or no interest has been shown in combining a specific upper lethal temperature and a thermal resistance range in an attempt to provide an enlarged description of the thermal response of various molluscan populations.

A study reported by Dzhamusova (1967) indicates that the temperature level for thermostability of muscle tissue of *Littorina squalida* is high and the range of thermostability is narrow as compared to *Littorina mandchurica* in which the temperature level of thermostability is low and the range is broad. Zhirmunsky (1967) presented the results of an extensive study on the thermostability of ciliated epithelial cells from 29 species of intertidal molluscs which range from the tropics to the arctic. The trend of the results indicates that the temperature level for thermostability decreases whereas the thermostability range increases as the latitudinal position of zoogeographic distribution of the species increases. A more detailed examination of a series of thermostability curves for the ciliated epithelial cells of tropical intertidal invertebrates enabled Zhirmunsky to place each species in its appropriate relative intertidal position. Those species which occur in the higher intertidal zone exhibited a higher temperature level of

thermostability while those from the lower intertidal zone exhibited a lower temperature level of thermostability. However, changes in thermostability ranges in relation to intertidal distribution could not be detected.

In the case of intact organisms, Read (1967) determined a thermal resistance line for samples of the tropical subtidal mollusc *Lima scabra*. Samples were collected in water 0.5 - 1 m deep. Water temperature during the collection period was about 26 C. Recalculation of Read's data gives an upper lethal temperature of 32 C for an exposure period of 5760 min and a regression coefficient for the line of -2.41. Results of the present study indicate that the blue mussel, which best approximates the vertical distribution of *Lima scabra*, has an upper lethal temperature of 28.40 C and a regression coefficient of -3.52 for the thermal resistance line.

In the zoogeographical distribution of molluscs, thermal response levels and upper lethal temperatures decrease with increasing latitude of species habitat when comparisons are conducted at the cellular and whole organism level. However, thermal response ranges and thermal resistance ranges broaden with increasing latitudinal distribution of species habitat when comparisons are conducted at the cellular and whole organism level.

Schlieper *et al.* (1960) presented thermal response lines for gill tissue of seven species of molluscs collected in different depths in the Mediterranean Sea. They reported a strong positive correlation between the temperature level for thermal resistance responses and depth distribution among the species. Higher temperature levels for thermal resistance response were obtained for those species inhabiting shallow waters whereas lower temperature levels for thermal resistance response were associated with those species inhabiting greater depths. However, differences in thermal response ranges could not be detected.

Reshöft (1961) presented data for cellular heat resistance in three species of subtidal lamellibranch molluscs which occur at different depths in the North Sea. Resistance lines have been established for each species by recalculation of those data. Comparisons among the three species indicate that the deepwater lamellibranch *Spisula solida* has the lowest cellular upper lethal temperature but the broadest thermal resistance range. The shallow water lamellibranch *Mytilus edulis* has the highest cellular upper lethal temperature but the narrowest thermal resistance range. A species of intermediate depth, *Modiolus modiolus*, has a cellular upper lethal temperature which is higher than that for *Spisula solida* but lower than the cellular upper lethal temperature of *Mytilus edulis*.

Sprague (1963) presents 24-hr upper lethal temperatures for four species of freshwater amphipod crustaceans which occur in different ecological conditions. He states that "the relative rank of the species according to their resistance agrees in general with expectations from their ecology". Those species which are found in waters which become relatively warm in summer have upper lethal temperatures which are higher than those for species which are typical of cooler waters.

The results of the present study show that upper lethal temperatures of intact animals are correlated with specific habitats within the intertidal zone of an estuary. The blue mussel, which occupies the lowest position in the intertidal zone, has a range of upper lethal temperatures which are lower than those for either the soft-shell clam or the ribbed mussel. The ribbed mussel which occupies the highest position in the intertidal zone has the highest range of upper lethal temperatures. The soft-shell clam which occupies a position in the intertidal zone immediately above the position occupied by the blue mussel has a range of upper lethal temperatures which is slightly above the range exhibited by the blue mussel. However, comparisons among widths of thermal resistance ranges indicate that the range for the blue mussel is considerably wider than it is for either the soft-shell clam or the ribbed mussel. The latter two species have thermal resistance ranges which are approximately equal in width.

Ecological significance of thermal responses

Adaptation is a word which has numerous meanings in biology. It broadly relates differences within and among organisms to environmental variation. For physiologists, the term refers to environmentally determined as well as to genetic variations. In this study, the term adaptation refers to any property of an organism which favours survival in a specific environment, particularly a stressful one. An adaptation permits maintenance of physiological activities and survival when the environment is altered with respect to one or more variables. Prosser (1964) states that those adaptations which are genetically determined can be separated from those which are environmentally induced by acclimation and ultimately by breeding experiments.

The adaptive significance of upper lethal temperatures and thermal resistance ranges pose a difficult problem for interpretation. Answers must be provided for two distinct questions before the adaptive significance of these capacities can be stated with any degree of certainty. Firstly, it is necessary to determine the specific proportion of a population which must be destroyed as a result of exposure to a potentially lethal stress before the results of a selection process are manifest in the subsequent generations. Added to this is the possibility that the selection process may be effective at a sublethal level of

thermal stress. Secondly, thermal tolerances should be defined for several levels of mortality response. Generally, the level of thermal tolerance is expressed for a 50% mortality response but it is conceivable that this is much too high a mortality response to consider in relation to adaptive significance.

It would be an arduous and time consuming task to determine the response level at which a selection process need operate to bring about an adaptive response to a potentially lethal thermal stress. Within the context of the present study, the best that can be achieved is to consider thermal resistance lines and upper lethal temperatures for lower levels of mortality response. Comparisons between thermal resistance lines resulting from 10% to 50% mortality responses indicate that the regression coefficients are homogeneous. Therefore, the absolute value of thermal resistance ranges are not altered as the mortality response level is changed from 10% to 50%. Upper lethal temperatures for a 5760 min exposure at higher temperatures are less than 0.7 degrees lower at a 10% mortality response level than at a 50% mortality response level. Such a decrease could of course be ecologically significant. However, further consideration of the adaptive significance of upper lethal temperatures and thermal resistance ranges will be considered for a 50% mortality response.

The level of thermal tolerance is not available for any mortality response level in the present study. The only statement that can be made relating upper lethal temperatures (LD50) to levels of thermal tolerance is that the thermal tolerance for a specific set of acclimation conditions would occur at a temperature equal to or below the stated upper lethal temperature for the specified acclimation conditions.

Within these limitations a plausible explanation for the adaptive significance of upper lethal temperatures (LD50) and thermal resistance ranges can be deduced from a consideration of the thermal conditions of a temperate intertidal zone.

The thermal conditions of such a zone can be resolved into two basic components which are related to the duration and intensity of thermal exposure. The first component is the series of daily temperature minima which is associated with seasonal changes which, in summer, is the period of extended warming of the species habitat (Fig. 2b). The second component is the diel thermal cycle which is associated with local conditions and in which excessive warming exists for only a few hours per cycle (Fig. 4). This component is imposed on the extended series of temperature minima. Survival of a population depends upon

the ability of individuals in the population to adapt to these components of the thermal conditions found in a temperate intertidal zone.

Upper lethal temperatures, as defined in this study, represent the ability of a population to resist those thermal levels which, when applied for several thousand minutes, constitute a potentially lethal stress. This ability varies among species and has been shown to be correlated to the extended warming conditions of the species habitat (Brett, 1952; Read, 1967).

Diel thermal conditions represent a somewhat different problem to a population. They represent local thermal conditions which are of short duration but of higher thermal intensities in relation to the extended warming conditions of the species habitat. These conditions can be most acute and represent stress which comes about too rapidly to be offset by acclimation. Survival under these conditions would necessitate a resistance range which would be a reflection of the relative intensities of the short-term thermal characteristics of the species habitat. Adaptation to daily thermal conditions in which the thermal level was slightly above the extended warming level would result in a relatively narrow thermal resistance range. Diel thermal conditions which were considerably higher than the extended warming level could act as a selective force for a much broader resistance range. This leads to the supposition

that thermal resistance levels for thermal exposures in excess of several days are established in response to the extended warming conditions of the species habitat while the thermal resistance range represents a response to the intensity of short-term thermal exposure. This scheme can be tested by considering the thermal response characteristics of blue mussels, soft-shell clams and ribbed mussels in relation to the thermal conditions of their habitats.

The blue mussel occurs almost exclusively in the lower portion of the intertidal zone. In this habitat, exposure to relatively high temperatures (>25 C), which are due to insolation, is usually infrequent and of short duration (< 300 min). The adaptative advantage of an upper lethal temperature much above 25 C would not be significant. However, a wide resistance range would provide an adaptive advantage in relation to resistance of thermal stress which resulted from short-term exposure at excessively high temperatures.

The soft-shell clam occupies a position in the intertidal gradient which is only slightly higher than the position of the blue mussel. Exposure to extended conditions would be similar to those experienced by the blue mussel. The resulting upper lethal temperature would be expected to be similar to that of the blue mussel.

Similarity of upper lethal temperatures should be observed for species which inhabit areas in which there is similarity of extended warming conditions. The results agree with this statement. The upper lethal temperatures for the soft-shell clam are only slightly higher than those for the blue mussel. The thermal resistance range of soft-shell clam is considerably narrower than that of blue mussel. Soft-shell clam is a benthic species which is usually covered by about 0.2 m of sand. The layer of sand acts as a thermal insulator which moderates thermal fluctuations before they reach the soft-shell clam. The adaptive advantage of a wide thermal resistance range would be minimal.

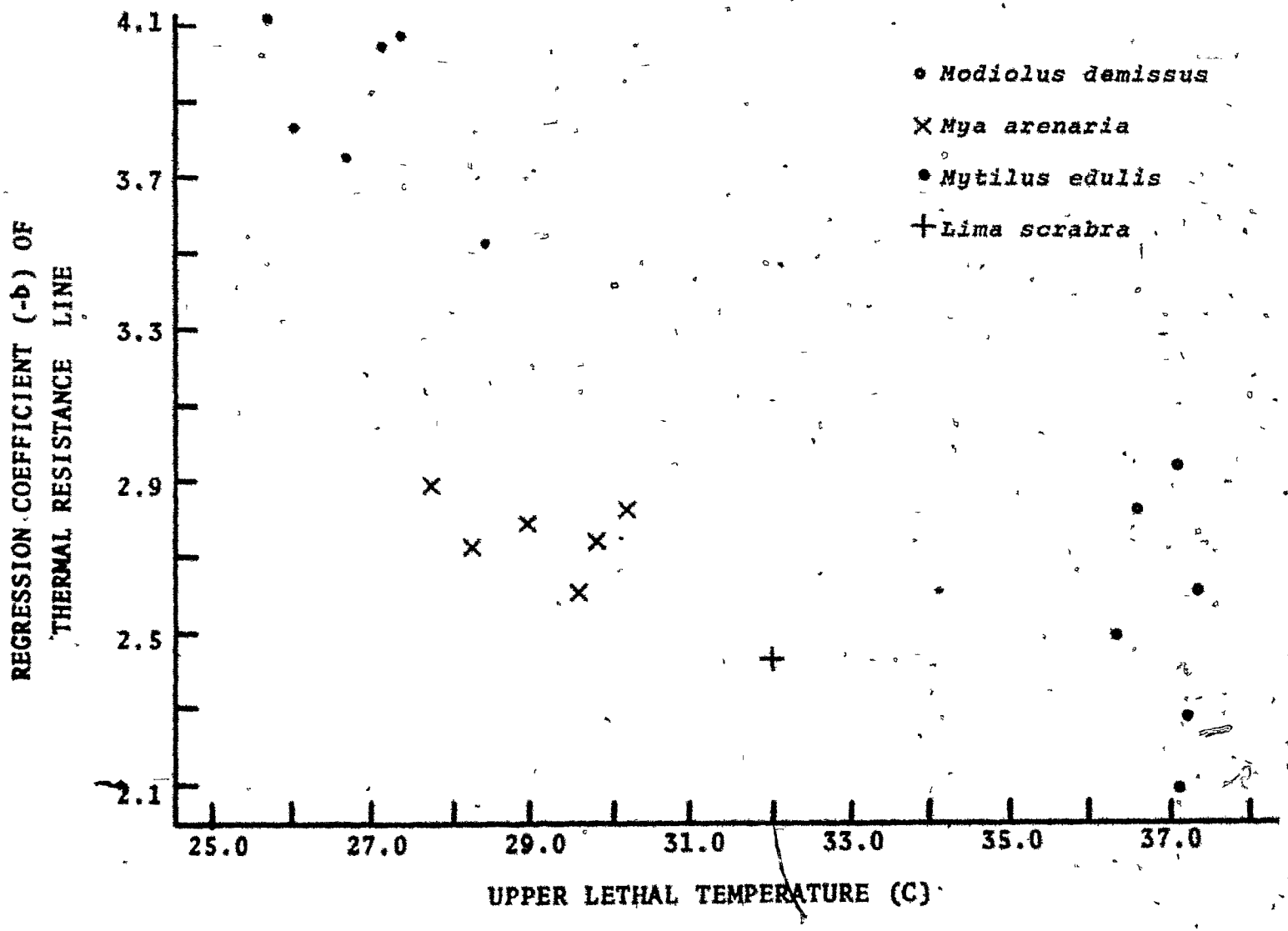
Ribbed mussels occupy a position in the intertidal zone which is considerably higher than the positions occupied by either the soft-shell clam or the blue mussel. Ribbed mussels experience thermal fluctuations which are relatively intense (>20° C) and of extended duration (>3 days). A high upper lethal temperature would represent an adaptive advantage for organisms living in this habitat. The results indicate that the upper lethal temperatures of the ribbed mussels are considerably higher than those determined for soft-shell clams and blue mussels. In the ribbed mussel the source of extended periods of elevated temperatures is atmospheric thermal conditions. An additional thermal effect is experienced by ribbed mussels during periods of direct insolation. The resulting highest short-term

temperatures experienced by ribbed mussels (30 C for 300 min) are only moderately higher than the temperature levels which represent the extended warming conditions of the species habitat. Under these conditions the adaptive advantage of a broad thermal resistance range would be minimal.

The information on thermal resistance, as related to the thermal conditions of the species habitat, leads to the presentation of a hypothesis which states that upper lethal temperatures are correlated with the extended warming conditions of the species habitat whereas thermal resistance ranges are correlated with the intensity and duration of diel thermal fluctuations within the species habitat.

A test of this hypothesis could be presented if an upper lethal temperature, as defined in this study, and a resistance line were available for another species from a different thermal environment. Read (1967) presented a thermal resistance line for samples of the tropical subtidal mollusc *Lima scabra* where water temperatures are about 26 C. Since this species is tropical in distribution, a relatively high upper lethal temperature would be predicted. Its subtidal distribution would enjoin a relatively stable thermal environment which would suggest a narrow resistance range. The resistance line has been recalculated to provide an upper lethal temperature and a regression coefficient for the thermal resistance line. The results are indicated in Fig. 30

Figure 30. Relationship among upper lethal temperatures and absolute value of regression coefficients of resistance lines for *Modiolus demissus*, *Mya arenaria* and *Mytilus edulis*. The data for *Lima scabra* came from Read (1967). Absolute values of regression coefficients are used in order to remove negative sign from all regression coefficients.


4.1
3.7
3.3
2.9
2.5
2.1
25.0
27.0
29.0
31.0
33.0
35.0
37.0
REGRESSION COEFFICIENT (-b) OF THERMAL RESISTANCE LINE
UPPER LETHAL TEMPERATURE (C)
Modiolus demissus
Mya arenaria
Mytilus edulis
Lima scrabra

and indicate general agreement with the hypothesis.

As in incidental note, it must be added that the capacity of an organism to tolerate or resist the thermal conditions of a habitat is probably not the only condition which determines habitat suitability and range.

In summary, the levels of higher temperatures at which thermal resistance is initiated in molluscs are correlated with the extended warming conditions of the species habitat. The thermal resistance range is correlated with the intensity and duration of short-term thermal fluctuations within each species habitat.

SUMMARY

For all species and acclimation combinations, time to 50% mortality decreased as the level of the test temperature was increased.

Time to 50% mortality for each test temperature decreased during a 28-day post-bioassay recovery period. This decrease has been attributed to the latent effects of a terminated thermal stress.

Thermal resistance lines are presented for zero-time and the 28th-day of post-bioassay recovery. Zero-time thermal resistance lines were curvilinear or linear, depending upon the temperature increment between acclimation and test temperature. The greater the increment, the greater the probability that the thermal resistance line will be curvilinear.

Upper lethal temperatures for a 5760-min exposure are presented for all species-acclimation combinations. Analysis of variance indicates that "species" and "acclimation temperatures" components are significant whereas the "acclimation salinity" component is not significant.

Regression coefficients of the thermal resistance lines were shown to be species-specific.

The range of upper lethal temperatures for each species in these bioassays is demonstrably higher than the mean ambient field temperatures.

Thermal resistance capacities are sufficient to compensate for the diel thermal fluctuations at the collecting sites.

Upper lethal temperatures and regression coefficients of thermal resistance lines are species-specific.

An explanation is presented for the adaptive significance of upper lethal temperatures and thermal resistance ranges.

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