

EFFECTS OF TEMPERATURE AND SALINITY ON
THERMAL DEATH IN
POSTLARVAL BROWN SHRIMP, Penaeus aztecus

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Prepared by

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OCTOBER 1970

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Partially Supported by the National Science Foundation
Sea Grant Program
Institutional Grant GH-59 to
Texas A&M University

Sea Grant Publication No. TAMU-SG-71-201

October 1970

ABSTRACT

Laboratory studies were conducted on the effects of temperature and salinity on resistance of postlarval Penaeus aztecus to lethally high temperatures. Tests were conducted on postlarval brown shrimp acclimated at three temperatures (24°, 29°, and 34°C) at nine lethal temperatures for each acclimation temperature (36.0° to 37.6°C; 38.0° to 39.6°C; 38.6° to 40.2°C). The results indicated that the upper incipient lethal temperature for shrimp acclimated at 24.0°C is between 36.6° and 36.8°C. No evidence of the upper incipient lethal temperature for postlarvae acclimated at 29° and 34° was found in the range of lethal temperatures used.

Acclimation to an increase in temperature by postlarval brown shrimp was studied, using increases in resistance to a lethal temperature as an indication of increasing acclimation. Acclimation to 34°C, after 8 days at 29°C, took place at a fairly steady rate for the first 3 days after transfer. No further acclimation took place between 3 and 7 days. A 10% decrease in salinity, concurrent with a temperature increase (29° to 34°C), caused a 24-hour increase in acclimation time to a total of 4 days.

Postlarval brown shrimp acclimated at the nine possible combinations of three acclimation temperatures (24°, 29°, and 34°C) and three acclimation salinities (5‰, 15‰, and 25‰), were tested for thermal resistance time at three test salinities (5‰, 15‰, and 25‰) for each of two lethal high temperatures. Resistance time increased with increasing acclimation temperatures and decreased with increasing lethal temperature.

Longer resistance times occurred at the higher test salinity (25‰) than at the other two salinities. The lower acclimation salinity (5‰) proved to be a better preparation for resisting lethal high temperatures, at all three test salinities, than either of the other two acclimation salinities. These results provide evidence of a new relationship between environmental salinity and the temperature tolerance of an estuarine organism. A reduction in the amount of work necessary for osmoregulation at 25‰, as compared to the two lower test salinities, is thought to explain the higher thermal resistance at higher test salinities. Improved thermal resistance at all test salinities after acclimation at 5‰ is considered to be an adaptation allowing the postlarvae to resist high temperatures when they are in low salinity bays.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the advice and assistance of the following people: Dr. D.V. Aldrich, Dr. K. Strawn, Dr. S.M. Ray, Dr. C.E. Gates, Dr. R.J. Freund, Mr. B. Lisenbe, Mr. J.S. Holland, and Mr. M. Karim. A special note of thanks to my wife, Jean, who provided valuable assistance in the preparation of this thesis.

These experiments were done at the Texas A&M University Marine Laboratory, Galveston, Texas. This study was partially financed by the National Science Foundation Sea Grant Program, Institutional Grant GH-59, made to Texas A&M University.

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INTRODUCTION

The brown shrimp, Penaeus aztecus, forms an important part of the commercial shrimp catch along the Texas coast. Its value as a commercial product has stimulated interest in the biology and ecology of this penaeid shrimp. In addition, interest in shrimp mariculture has focused attention on brown shrimp as a potential organism for pond culture.

The effects of different temperatures and salinities upon brown shrimp postlarvae is of particular interest because of the life history of this species. Brown shrimp spawn offshore in the open sea. After reaching the postlarval stage, the young brown shrimp enter bays and estuaries, penetrating to the shallowest areas of these bodies of water. Thus, the young shrimp go from an area of relatively stable temperatures and salinities (the sea) to one in which both factors may fluctuate widely (the bays and estuaries).

A knowledge of how temperature and salinity affect brown shrimp postlarvae can aid in interpreting the ecology of these animals during this phase of their life cycle. Information on the effects of environmental factors on an animal is a basic requirement for the successful

The citations on the following pages follow the format of the journal "Biological Bulletin".

husbandry of that animal. Therefore, a knowledge of the effects of temperature and salinity on brown shrimp postlarvae could be of value to mariculturists planning to raise brown shrimp.

Increasing use of bays and estuaries as sources of cooling water for power plants may lead to discharges of heated waters into these bodies of water. Information on the effects of high temperatures on postlarval brown shrimp could aid in evaluating the effects of warm-water discharges on these animals.

The purpose of this investigation was to study the effects of different temperature-salinity combinations on postlarval brown shrimp which were passing from the sea into the bays.

REVIEW OF THE LITERATURE

Salinity has an important influence upon marine and estuarine animals through its effect upon ionic- and osmoregulation. Crustaceans generally regulate the ionic composition of their body fluids, and in addition, many have the ability to osmoregulate (Robertson, 1960). There is evidence that Penaeus aztecus has the ability to osmoregulate.

Williams (1960) used freezing-point determinations on blood of juvenile and adult P. aztecus to study their osmoregulatory ability at different salinities. He found that at temperatures of 25°-32° C, the blood was hypotonic to salinities above 30‰ and hypertonic to salinities below 30‰. Osmoregulation was reportedly impaired at low temperatures (8°-9° C). McFarland and Lee (1963) found adult brown shrimp to be isosmotic to waters with salinity concentrations of 830 to 850 milliosmols (27‰-28‰, approximately) at temperatures of 27°-28° C. The shrimp were hyposmotic to salinities higher than 27‰-28‰ and hyperosmotic to lower salinities.

It is well known that temperature also exerts a profound effect upon poikilothermal animals. Brett (1956) has effectively summarized the various effects of temperature upon poikilothermal animals:

"Temperature sets lethal limits to life; it conditions the animal through acclimation to meet levels of temperature that would otherwise be intolerable; it governs the rate of development; it sets the limits of metabolic rate within which the animal is free to perform; and it acts as a directive factor resulting in the congregation of fish within given thermal ranges, or movements to new environmental conditions."

The range of temperatures at which an animal can live without suffering any lethal effects due to temperature is defined as the "zone of tolerance" (Brett, 1956). Temperatures which will eventually prove lethal are termed the "zone of resistance".

Several theories have been advanced to explain heat death in poikilothermal animals. Fry (1958) and Ushakov (1964) have reviewed the work relating melting point of lipids to thermal death. While in some cases feeding animals lipids with high melting points increased resistance to high temperatures, this was not always the case (Ushakov, 1964). Some proponents of this point of view have theorized that the number of double bonds in the lipid is the important factor rather than melting point (Hoar and Cottle, 1952).

Christophersen and Precht (1953) proposed the theory that thermal resistance and acclimation are a result of changes in the water content of the cells. The amount of water bound to protein molecules within a cell controls the thermal stability of the molecule, according to this theory. An increase in the amount of free water

(and a decrease in bound water) in a cell causes decreased thermal resistance (Christophersen and Precht, 1953). Baldwin's (1954) discovery of increasing heat resistance with increasing desiccation in some insects would seem to support this theory. Differences in free to bound water ratios have been reported in frogs acclimated to different temperatures, according to Fry (1958). However, as Fry points out, desiccation is not the only factor involved in resistance in insects, and differences in free-bound water ratios have not been reported in all cases of animals acclimated to different temperatures.

The concept of heat denaturation of proteins in cells as a cause of heat death has been reviewed by Read (1964) and Ushakov (1964). Both authors concluded that while the temperature necessary for denaturation of some proteins is increased with increased acclimation temperature, the temperatures at which denaturation occurs are usually well above the temperatures at which heat death occurs in the whole organism. Ushakov (1964) theorizes that "decomplexation" of protoplasmic proteins rather than changes in the protein molecules themselves may be the cause of thermal death. However, as Ushakov admits, there is no evidence to support this theory.

It appears that while the theories discussed here each have some supporting evidence, there are also difficulties in each one. Thus,

the question of the exact process of thermal death at the cellular level remains unresolved.

Bowler (1963b) found that in crayfish, lethal temperatures affected ionoregulatory ability, bringing about an imbalance in sodium and potassium ions between the blood and body fluids. He proposed that heat death was due to a loss of nervous coordination resulting from this imbalance and "the direct effect of heat on the neuro-muscular system". A study of the effects of sublethal heat shock on ionic regulation in goldfish by Heinicke and Houston (1965) found deviations in ionoregulation followed heat shock. However, the blood concentrations of sodium did not decrease, as reported by Bowler for the crayfish.

A lethal temperature study on the longear sunfish (Neill et al., 1966) indicated that the fish could be separated into physiological populations in which death was due to different lethal effects. They based this conclusion on differences in variances of logarithms of resistance time, with high variance indicating more than one lethal effect. Evidence of six lethal effects in channel catfish was reported by Allen and Strawn (1967). The results of these studies indicate that there may be more than one cause of heat death in poikilotherms.

An animal subjected to a lethal temperature may live for a period of time at that temperature. The length of time it lives is referred to as the resistance time (Brett, 1956). When the logarithms of the medians of resistance times at different lethal temperatures are plotted against the lethal temperatures, the medians form a linear series (Brett, 1956). At a certain lethal temperature, which varies with the species of animal and the acclimation temperature, an abrupt change in the slope of a line fitted to the medians occurs. Brett (1956) considers this abrupt change to indicate a change from resistance to tolerance of the temperature, and defines this point as the upper "incipient lethal temperature". The length of the resistance time of an organism is influenced by the lethal temperature (Prosser and Brown, 1961) as well as by many other factors.

Acclimation temperature is one such factor. As Brett (1956) and Fry (1957) have noted, the temperature at which an animal has lived prior to exposure to a lethal temperature has a direct effect upon resistance time at the lethal temperature. Numerous studies on fish reveal an increase in resistance time at a given lethal temperature with increasing acclimation temperatures (Brett, 1956). Investigations on the crayfish (Spoor, 1955), the lobster (McLeese, 1956), and the blue crab (Tagatz, 1969), among others, indicate that crustaceans show similar responses to acclimation temperature.

In most fish, gain of heat tolerance, indicating acclimation after a temperature change, is fairly rapid for increasing temperatures. Brett (1956) states that acclimation in fish is completed in about 24 hours at temperatures above 20° C.

In crayfish (Orconectes rusticus) transferred from 24° to 4° C, heat tolerance lost during 13 days at 4° C was regained in 24 hours after transfer back to 24° C (Spoor, 1955). Bowler (1963a) found that acclimation was complete at 3 days after a change from 8° to 25° C in the crayfish, Astacus pallipes. The isopod Asellus intermedius, after undergoing a change from 20° to 30° C, completed its acclimation in 3 days, while the amphipod Hyaella azteca took 2 days to acclimate to a change from 14° to 20° C (Sprague, 1963). In the amphipod Gammarus fasciatus, acclimation to 20° from 15° C took 1 day in males and 2 days in females (Sprague, 1963). McLeese (1956) found that in the American lobster, survival time increased only slightly for the first 10 days after a change from 14.5° to 23.0° C. Acclimation was comparatively rapid from 10 to 22 days, with acclimation being essentially complete after 22 days.

Several studies have been conducted on the effects of salinity upon resistance time in various fishes and crustaceans. McLeese (1956) found that in lobsters, the upper lethal temperature decreased with reduced salinities. He reported that resistance to lethally low

salinities was affected by the acclimation salinity, but that the effect depended on temperature acclimation.

In the copepod, Tigriopus fulvus, increases in salinity are paralleled by an increase in thermal tolerance (Ranade, 1957). Todd and Dehnell (1960) found that resistance to lethal temperature was better at higher salinities in the grapsoid crabs Hemigrapsus nudus and H. oregonensis.

Upper lethal temperatures were found to be highest at isosmotic salinities in the cyprinodontid fish Fundulus heteroclitus and F. diaphanus (Garside and Jordan, 1968). Ten species of salt- and freshwater-marsh fishes were tested for thermal resistance at various salinities by Strawn and Dunn (1967). They found that the optimum salinity for survival decreased for freshwater fishes and increased for salt-marsh fishes with decreasing lethal temperatures.

Tagatz (1969) reported that blue crabs (Callinectes sapidus) were less tolerant to temperature extremes at low salinities. Lewis and Hettler (1968) found that salinity did not markedly affect survival at high temperatures in the menhaden, Brevoortia tyrannus.

The life cycle of the brown shrimp has been reviewed by Williams (1965) and Baxter (1962). The adult Penaeus aztecus spawn in the open sea. After hatching, the larvae undergo several stages, eventually developing into the postlarval stage. At this stage, P. aztecus

enter estuaries and bays, moving to the shallow areas of these bodies of water (Williams, 1965). After a period of rapid growth in the bays, brown shrimp return to the open sea to complete their life cycle. In Galveston Bay, Baxter (1962) found that postlarval brown shrimp enter in two main groups. Most enter in the spring (February to May) with another, smaller influx in the fall. During their postlarval stage, brown shrimp move from the sea, an area of relatively stable temperature-salinity conditions, to the bays, where considerable fluctuations of both factors may occur.

Gunter (1956, 1961) has documented the penetration of Penaeus aztecus postlarvae into areas of very low salinity. He has concluded that survival of postlarval brown shrimp is dependent on their entering low salinity waters, stating that (Gunter, 1961): "small shrimp are not killed or precluded by high salinity as if it were poison, they simply do not do well in it, for reasons unknown". More recently (Gunter et al., 1964), Gunter and others have reaffirmed their belief that salinity per se has a limiting effect on distribution of young shrimp. Gunter et al. (1964) have reported that young (juvenile) brown shrimp are found at salinities from 10‰-30‰, with more found at salinities below than above 20‰.

Several laboratory investigations of the effects of temperature and salinity upon growth and survival of postlarval brown shrimp have

been conducted. Zein-Eldin (1963) subjected postlarval brown shrimp to five salinities ranging from 2‰ to 40‰ at a temperature range of 24.5° to 26° C. She observed excellent survival at all salinities tested, and no significant differences in growth rates at different salinities.

Another investigation (Zein-Eldin and Griffith, 1966) studied growth and survival of Penaeus aztecus postlarvae at different temperatures. The temperatures used were between 15° and 35° C, and salinity was maintained at 25‰. Growth was observed to increase with increasing temperature up to 32.5° C. Survival, however, decreased with increasing temperatures above 25° C.

Zein-Eldin and Aldrich (1965) found that salinity had little effect on survival or growth of brown shrimp postlarvae except at extreme temperatures. They found reduced tolerance to lower salinities (less than 15‰) at temperatures below 15° C.

MATERIALS AND METHODS

General Procedure

This investigation consisted of three experiments. These were the lethal temperature experiment, the acclimation experiment, and the temperature-salinity experiment.

Lethal Temperature Experiment

Postlarvae held at three acclimation temperatures were tested at nine lethal temperatures. The purpose of this experiment was to obtain information on thermal resistance time at different lethal temperatures. Lethal temperatures for subsequent experiments were chosen on the basis of information gained in this experiment.

Acclimation Experiment

After acclimation at one temperature, postlarvae were transferred to a higher temperature. Periodic tests of thermal resistance were used to determine the time necessary for the postlarvae to acclimate to the higher temperature. This experiment was designed to give information on the length of time necessary for postlarvae to complete acclimation to a higher temperature.

Temperature Salinity Experiment

After acclimation to nine combinations of three temperatures and three salinities, ten postlarvae from each combination were tested for thermal resistance time at three salinities for each of two lethal temperatures. The purpose of this experiment was to discover the effects of temperature and salinity on postlarval brown shrimp by observing differences in resistance time.

Equipment

Acclimation Equipment

The postlarvae were acclimated in rectangular 6-gallon aquaria which were placed inside 88-gallon acclimation tanks (Fig. 1). The larger tanks, which were used as water baths for temperature control for the aquaria, are constructed of plywood covered with polyester resin. These tanks are completely enclosed, and are insulated with 1/2-inch polyurethane foam between double walls of plywood.

Temperature is controlled in the 88-gallon tanks by a contact thermometer which activates an electrical relay. In tanks in which the desired temperature was above room temperature, the relay operated two 250 W immersion heaters. In those tanks in which the desired temperature was below room temperature, the relay also



Figure 1. (Top) Acclimation tank with two aquaria in place. Hand points to the polyurethane float.

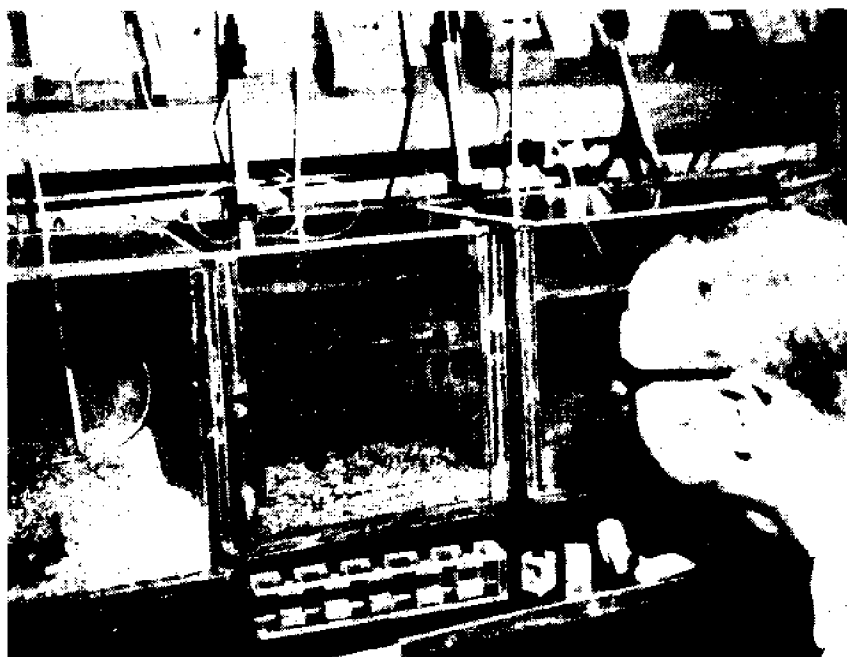


Figure 2. (Bottom) Lethal-test tank with 10 cages inside. A holder with five cages, and two individual cages, are shown in front of the tank.

operated a cooling coil in the tank. Air stones in the 88-gallon tanks circulated the water to prevent thermal stratification.

Two aquaria were placed in each 88-gallon tank, resting on two 8 x 8 x 16-inch cinder blocks (Fig. 1). In order to insure temperature control, the aquaria were emersed in the water bath so that the water level in the aquarium was slightly below that of the water bath. Using this method, it was possible to maintain temperatures within $\pm 0.1^{\circ}$ C of the desired temperature within the aquaria.

Each aquarium was equipped with an under-gravel filter covered first by a layer of crushed oyster shell and then by a layer of blasting sand. A cover of polyethylene film was placed on the surface of the water to prevent escape of the postlarvae. An air stone placed in the middle of each tank provided aeration and circulated the water. A circular float of polyurethane foam covered an opening in the cover which allowed air to escape (Fig. 1).

A photoperiod of 12 hours of light and 12 hours of darkness was maintained in all acclimation tanks. Tork model 7300 timers were used to control the photoperiod. A single 30 W standard cool white fluorescent bulb provided illumination in the tanks during the light period.

The seawater used in all experiments was obtained from the Gulf of Mexico. It was stored in a large outside storage tank

for several days before being used, in order to allow sediments to settle out. When necessary, the seawater was diluted with distilled water to reduce the salinity. Salinity was determined with a Goldberg refractometer (American Optical Co.).

Lethal Test Equipment

Thermal resistance tests were conducted in 8-gallon tanks constructed of polyester resin covered plywood. One end of the tank consists of a plexiglas sheet, as does the cover (Fig. 2). Temperature was controlled in the same manner as in the acclimation tanks. Two air stones in each tank aerated and circulated the water.

Postlarvae were placed inside small cages during the thermal resistance tests in order to keep them within easy view of the observer (Fig. 2). These cages were constructed of 1/2-inch plexiglas. Plastic window screen was used to cover two sides to allow water to circulate through the cages. A 1/2-inch opening in the top, which was closed with a 00 rubber stopper, allowed access into the cage. The outside measurements of the cages are approximately 2 x 2 x 1 inches. Five cages were placed in a plexiglas holder (Fig. 2, foreground) which facilitated transfer of the shrimp to the lethal test tanks. Inside the tanks, two holders were placed on a plexiglas rack (Fig. 2).

Postlarvae were transferred from the acclimation aquaria to the cages using a bulb pipette with a large opening. The shrimp were first netted and placed in a large finger-bowl emersed in the water bath. They were then transferred to the cages, which were placed inside the acclimation aquaria.

In the thermal resistance tests, all postlarvae were watched continuously and death times were recorded to the nearest 0.1 minute. Cessation of coordinated movement of the appendages was used as the indication of death. This is a common method of determining time of death in thermal death experiments on crustaceans (Bowler, 1963a; McLeese, 1956; Sprague, 1963).

Specific Procedure

Sources of Postlarvae

Postlarvae for this investigation were collected on the beach at Galveston, Texas near the 61st street fishing pier (station #1) (Fig. 3), or on a sand flat in the entrance to Galveston Bay, just west of the south jetty (station #2) (Fig. 3). Station 1 was used in good weather because of its nearness to the Texas A&M Marine Laboratory. Postlarvae were collected at station 2 when weather conditions made the surf at station 1 too rough for towing.

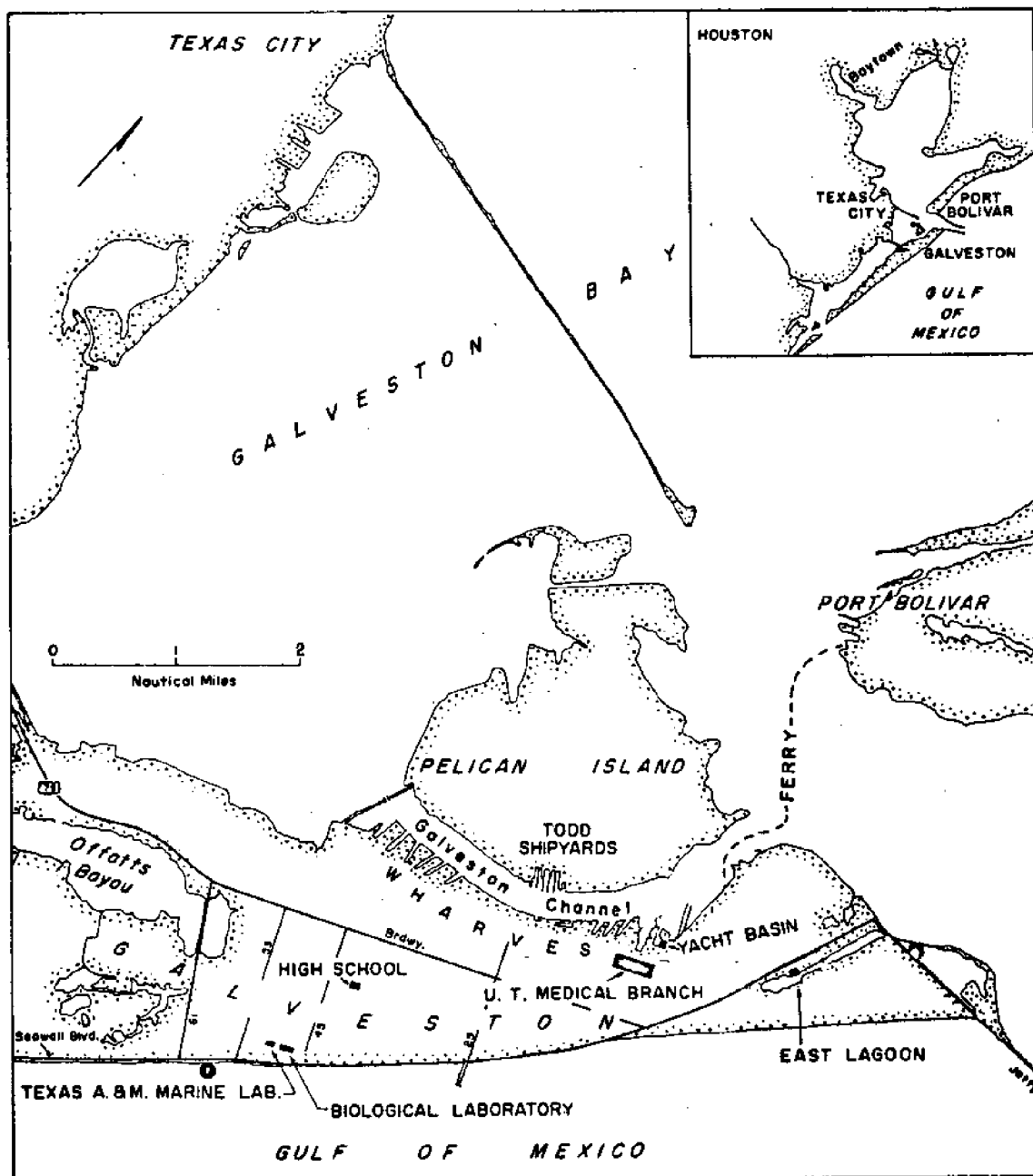


Figure 3. Map showing the locations of the two collecting stations. Black circle (●) to the left of the Texas A&M Marine Laboratory is station #1. The circle to the right of the East Lagoon is station #2.

Postlarvae were collected using a hand-drawn beam trawl as described by Renfro (1962). The catch, and approximately 20 liters of seawater from the collection site, was transported to the laboratory in a styrofoam ice chest and aerated by a battery-operated air pump. Postlarvae were separated from other organisms in the catch by using a small square of plastic window screen to catch the shrimp.

Lethal Temperature Experiment

On February 25, 1970, postlarval brown shrimp for the lethal temperature experiment were collected at station 1. The water temperature at the time was 13.7° C and the salinity was 28‰. One hundred and twenty postlarvae were placed in two aquaria at each of the three acclimation temperatures, 24.0°, 29.0°, and 34.0° C. The acclimation salinity was 28‰, the same as the field salinity. No information on the time necessary for acclimation to a higher temperature was available for brown shrimp, so a period of 8 days was arbitrarily chosen.

After 8 days of acclimation, 10 shrimp from each acclimation temperature were tested at each of nine lethal temperatures. The lethal temperatures at which animals from each acclimation temperature were tested are given in Table 1. Salinity in all lethal test tanks was 28‰.

TABLE 1
Acclimation and lethal temperatures used in the
lethal temperature experiment

Acclimation Temperature	Lethal temperatures (° C)									
24	36.0	36.2	36.4	36.6	36.8	37.0	37.2	37.4	37.6	
29	38.0	38.2	38.4	38.6	38.8	39.0	39.2	39.4	39.6	
34	38.6	38.8	39.0	39.2	39.4	39.6	39.8	40.0	40.2	

Acclimation Experiment

Postlarvae for the acclimation experiment were collected on March 10, 1970. The water temperature at the time of collection was 17.2° C, and the salinity was 25‰. For this experiment, 300 shrimp were acclimated at 29.0° C and 25‰ for 8 days. Half of the remaining postlarvae were then transferred to two aquaria containing 15‰ water at 34.0° C, while the others were transferred to 25‰ water, also at 34.0° C. The purpose of using two salinities was to determine if a concurrent reduction of salinity affected acclimation to a higher temperature, as opposed to a simple change to a higher temperature.

Thermal resistance tests were conducted at just before transfer ("0" hours) and 3, 12, and 24 hours after transfer. After the 24-hour test, postlarvae were tested at 24-hour intervals, through 72 hours. After the experiment had begun, it was learned that a die-off of barnacles and annelids in the seawater tank had contaminated the seawater used in some of the acclimation aquaria. The possibility of this affecting thermal resistance led to a decision to terminate the experiment after 72 hours.

Due to the problems encountered in the acclimation experiment, it was decided to repeat the entire experiment. Postlarvae for this

purpose were captured at station 2 on March 24, 1970. The water temperature was 19.7° C and the salinity was 28‰ at the collecting site. Acclimation temperatures were the same as in the first experiment, but salinities of 18‰ and 28‰ were used.

The thermal resistance tests were conducted at the same time intervals as in the first experiment except that after 24 hours tests were conducted at 24-hour intervals through 168 hours. The experiment was limited to 7 days (168 hours) because of restricted acclimation aquarium space. The lethal temperature was 39.8° C in this experiment. Shrimp transferred to 18‰, were tested at that salinity, and those at 28‰ were tested at 28‰.

Temperature Salinity Experiment

Postlarvae for this experiment were captured at station 1 on April 9, 1970. The salinity at the time was 29‰, and the water temperature was 20.6° C.

Eighty postlarvae were placed in each of two aquaria at each of the nine temperature-salinity combinations. The acclimation temperatures and salinities are given in Tables 2-4. Two replications of each acclimation combination were used. The postlarvae were allowed to acclimate for 6 days prior to thermal resistance tests. An acclimation period of 6 days was selected on the basis of the

TABLE 2

Acclimation and test conditions in the temperature salinity experiment (postlarvae acclimated at 24.0° C). Ten shrimp from each set of acclimation conditions were tested at each of the test conditions.

Acclimation conditions		Test conditions	
Temperature	Salinity	Temperature	Salinity
		36.6	5
24.0	5	36.6	15
		36.6	25
24.0	15		
		37.0	5
24.0	25	37.0	15
		37.0	25

TABLE 3

Acclimation and test conditions in the temperature salinity experiment (postlarvae acclimated at 29.0° C). Ten shrimp from each set of acclimation conditions were tested at each of the test conditions.

Acclimation conditions		Test conditions	
Temperature	Salinity	Temperature	Salinity
		38.2	5
29.0	5	38.2	15
		38.2	25
29.0	15		
		39.2	5
29.0	25	39.2	15
		39.2	25

TABLE 4

Acclimation and test conditions in the temperature salinity experiment (postlarvae acclimated at 34.0° C). Ten shrimp from each set of acclimation conditions were tested at each of the test conditions.

Acclimation conditions		Test conditions	
Temperature	Salinity	Temperature	Salinity
		39.0	5
34.0	5	39.0	15
		39.0	25
34.0	15		
		39.8	5
34.0	25	39.8	15
		39.8	25

results of the acclimation experiment. At 6 days, the postlarvae would be well beyond the period of fairly rapidly increasing thermal resistance characteristic of the first 3 days of acclimation. There were not enough thermal test tanks available to conduct all of the resistance tests at the same time, so the starting times of some of the acclimation combinations were delayed in order to allow the same period of acclimation of all shrimp, yet spread out the tests over several days. Ten postlarvae were tested at each of three salinities and two lethal temperatures. The test conditions are summarized in Tables 2-4.

Acclimation temperatures for this experiment were 24°, 29° and 34° C, as in the lethal temperature study. Acclimation salinities were 5‰, 15‰ and 25‰. These temperatures and salinities were chosen because they approximate the range of these factors in the bays when postlarval brown shrimp are present. Two lethal temperatures were chosen, from information obtained in the lethal temperature experiment, for each acclimation temperature. Lethal temperatures were chosen so as to have resistance times in the range of 10-100 minutes at one lethal temperature, and 100-1,000 minutes at the other. This was to give a wide range of resistance times, and to permit the expression of as many physiological death mechanisms (as described by Neill et al., 1966) as possible. Death times in

excess of 10 minutes are desirable because experience has indicated that shorter death times may tend to mask the effects of different factors due to the overwhelming effects of the lethal temperature. Resistance times in excess of 1,000 minutes involved lengths of time which were deemed impractical for this experiment. It was decided to test postlarvae from each temperature-salinity combination at 5‰, 15‰ and 25‰ salinity at each lethal temperature. This was to study the effects of different salinities on resistance to a lethal temperature.

High mortality of postlarvae being acclimated at 24° C - 5‰ occurred between the 4th and 6th days of acclimation. Mortality in the first replication was close to 100%, and tests for that replication were not done. Mortality in the second replication was high in one aquarium, but survival was good in the other. Enough postlarvae survived in this replication to allow completion of all resistance tests. A second attempt to do the first replication also failed, almost total mortality occurring between the 5th and 6th days of acclimation. No further attempt was made to complete the missing replication.

Survival at the other two salinities at 24° C acclimation temperature was good, as was that in all other 5‰ acclimation salinities

(a complete listing of percent survival in all aquaria after 6 days acclimation is given in Table 5). The causes of the high mortality at 24° C and 5‰ are not known.

Presentation of Data

The results of the lethal temperature experiment are plotted on a semi-logarithmic scale in Figure 4. This is the usual way of presenting resistance time data, and stems from the theory that such data is log-normal in distribution (Strawn and Dunn, 1967).

The results of the acclimation experiment are also plotted on a semi-logarithmic scale (Fig. 5 and 6). The lines interconnect mean resistance times and were fitted by eye.

Data from the temperature-salinity experiment is presented in several ways. Semi-log plots compare resistance times at the three test salinities for each acclimation temperature salinity in Figures 7-12.

Frequency diagrams (Fig. 13-15) were made by ranking the mean resistance time of the factor being plotted (acclimation or test salinity) as 1, 2 or 3 on the basis of longest, intermediate, or shortest resistance time. The results are plotted as number of means at each rank.

TABLE 5

Mortality of postlarvae after 6 days acclimation in the
 temperature salinity experiment. Number of
 postlarvae/aquarium at beginning = 40.

Acclimation conditions	Replication	Aquarium	Percent mortality
24° C, 5‰	1	1	95.0
		2	93.0
	2	1	2.5
		2	45.0
24° C, 15‰	1	1	5.0
		2	5.0
	2	1	2.5
		2	0.0
24° C, 25‰	1	1	7.5
		2	5.0
	2	1	2.5
		2	7.5
29° C, 5‰	1	1	5.0
		2	7.5
	2	1	7.5
		2	7.5
29° C, 15‰	1	1	7.5
		2	10.0
	2	1	5.0
		2	40.0*

TABLE 5 (continued)

Acclimation conditions	Replication	Aquarium	Percent mortality
29° C, 25‰	1	1	2.5
		2	5.0
	2	1	2.5
		2	2.5
34° C, 5‰	1	1	2.5
		2	7.5
	2	1	2.5
		2	2.5
34° C, 15‰	1	1	5.0
		2	2.5
	2	1	2.5
		2	2.5
34° C, 25‰	1	1	5.0
		2	7.5
	2	1	0.0
		2	42.5*

*high mortality due to failure of air supply overnight.

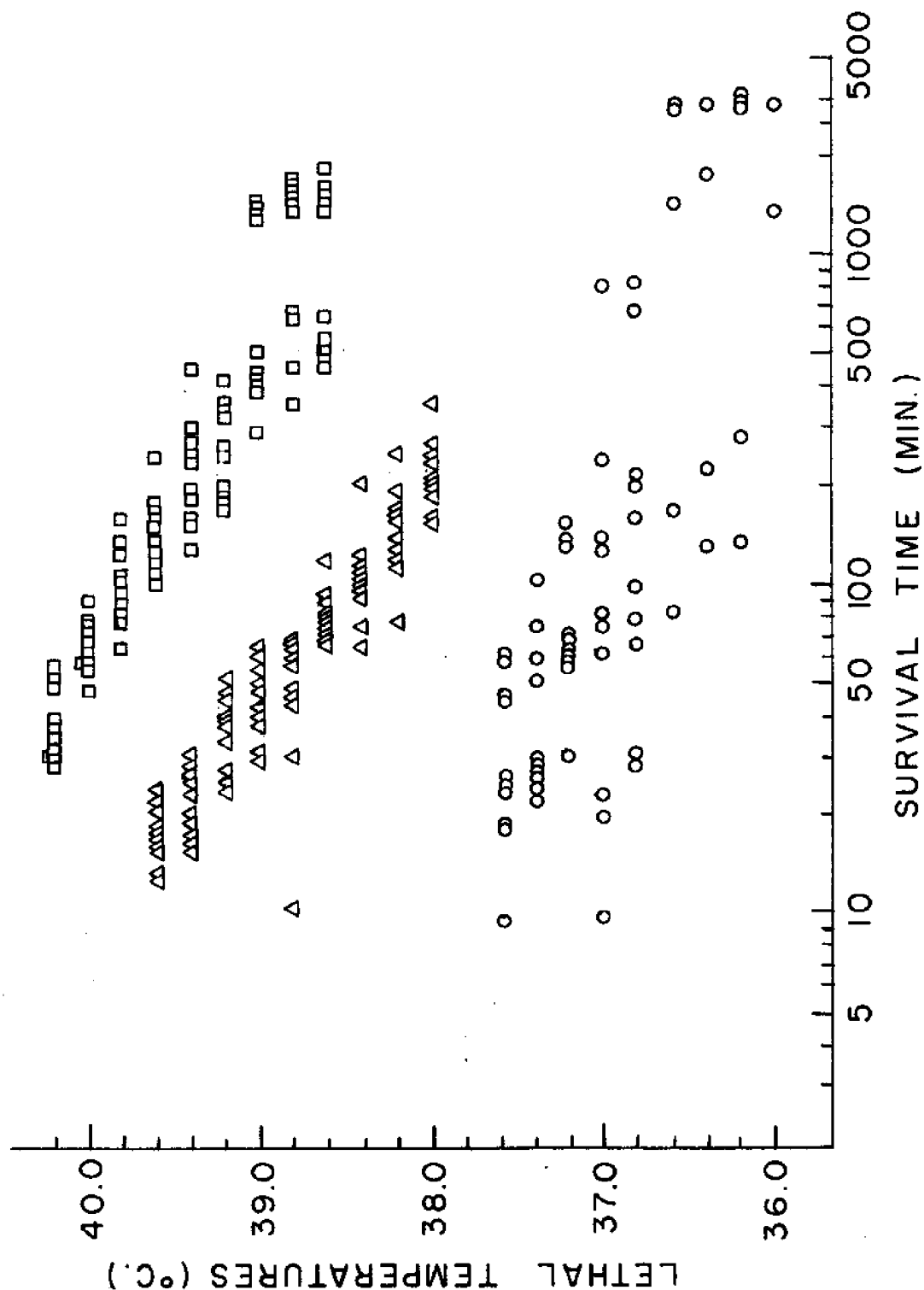


Figure 4. Survival (thermal resistance) times at different lethal temperatures of postlarvae tested in the lethal temperature experiment. The shrimp were watched continuously for 900 minutes, after which the survivors were checked at periodic intervals. Death times of postlarvae which died after 900 minutes were recorded as the time of the periodic check at which they were found dead. Tests on postlarvae acclimated at 24°C were ended at 3,243 minutes.
 (□ = one postlarva acclimated at 34°C; △ = one postlarva acclimated at 29°C; ● = one postlarva acclimated at 24°C)

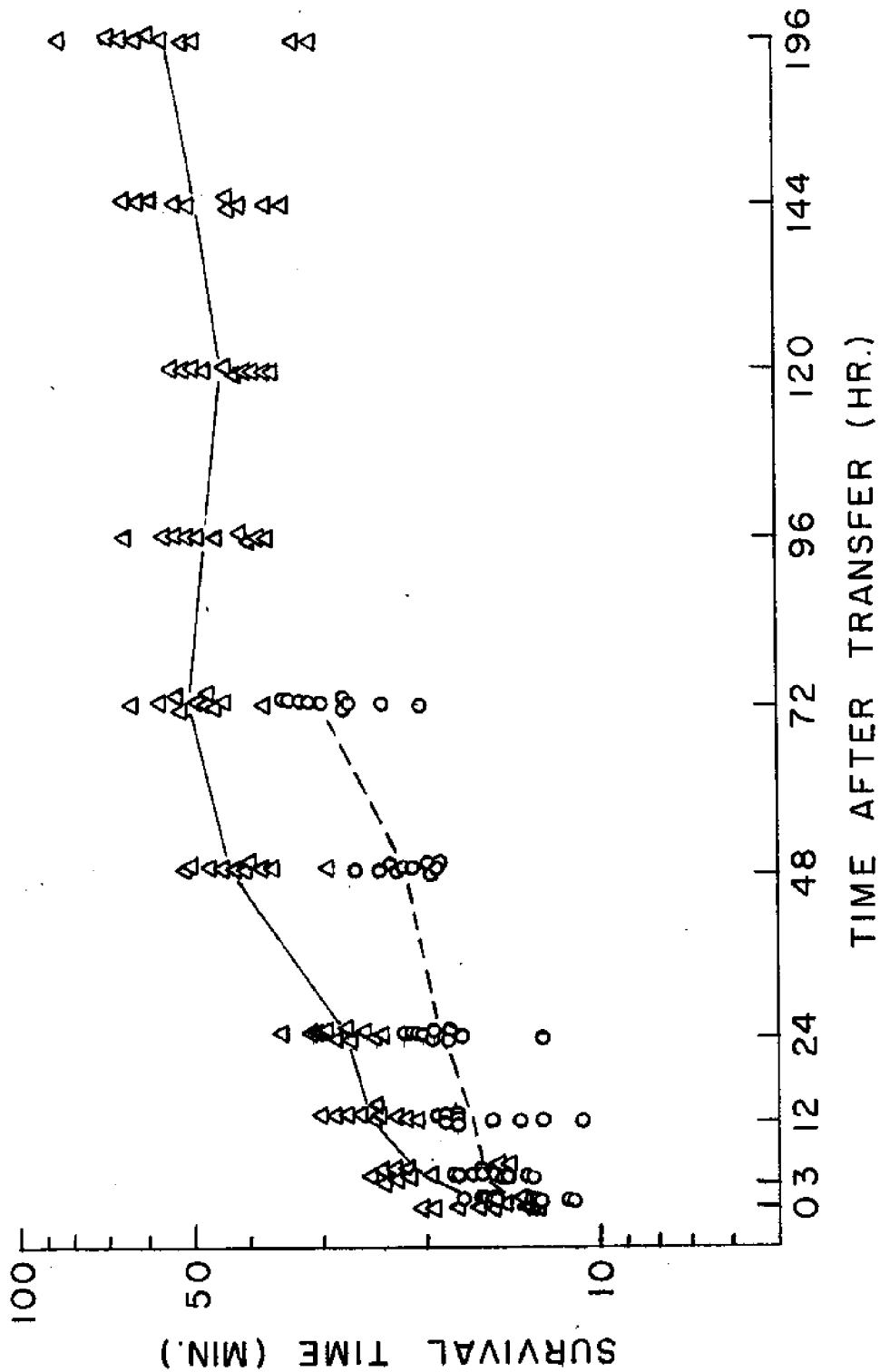


Figure 5. Survival (thermal resistance) times of postlarvae tested at different times after transfer from 29° to 34°C in the acclimation experiment. These shrimp did not undergo a salinity change at the same time as the temperature change. The lines interconnect mean resistance times, and were fitted by eye. (○ = one postlarva tested at 40.0°C and 25%; Δ = one postlarva tested at 39.8°C and 28%)

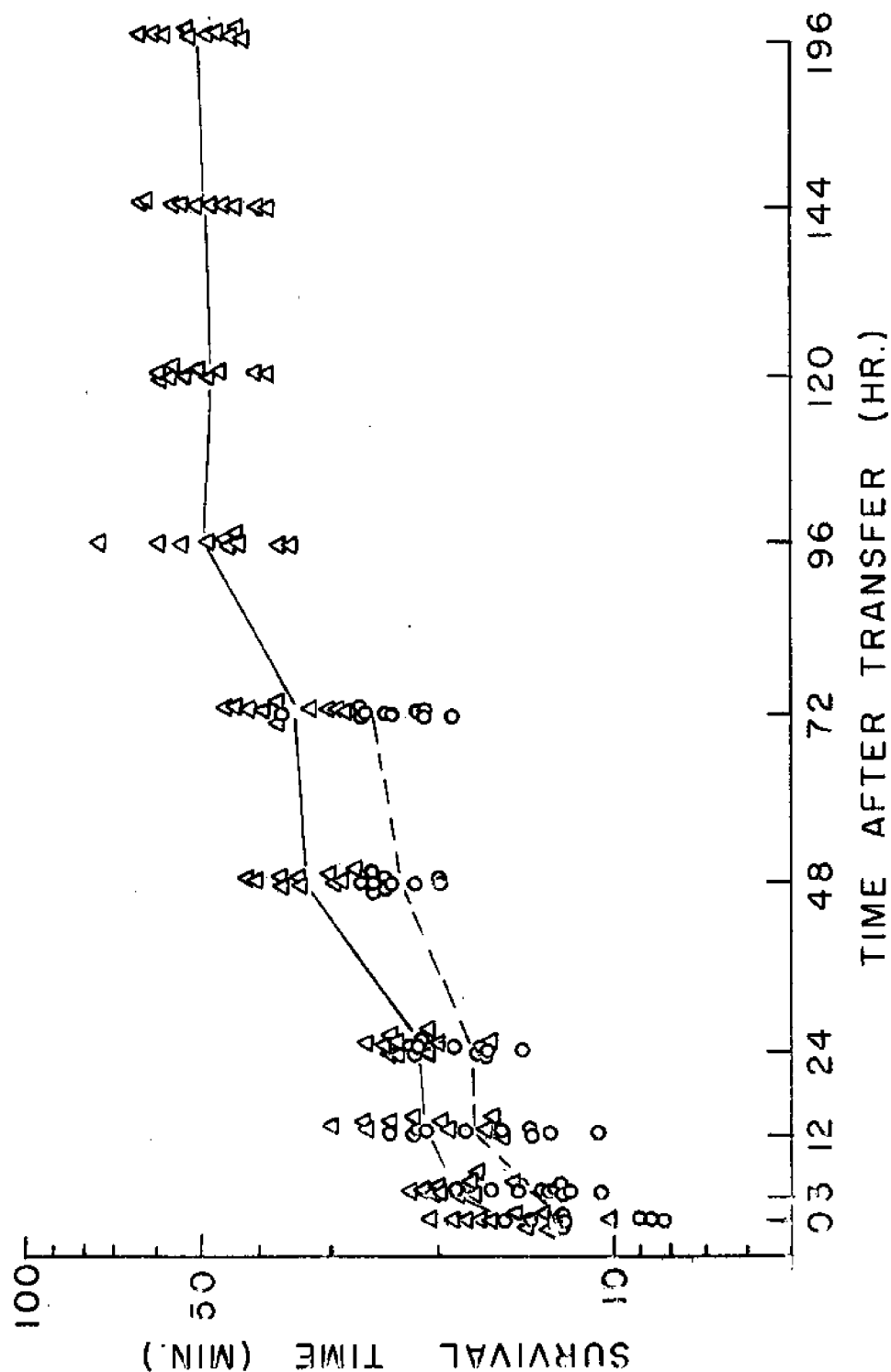


Figure 6. Survival (thermal resistance) times of postlarvae tested at different times after transfer from 29° to 34°C in the acclimation experiment. These shrimp underwent a 10% reduction in salinity concurrent with the acclimation temperature increase. The lines interconnect mean resistance times, and were fitted by eye.
 (○ = one postlarva tested at 40.0°C and 15%; △ = one postlarva tested at 39.8°C and 18%)

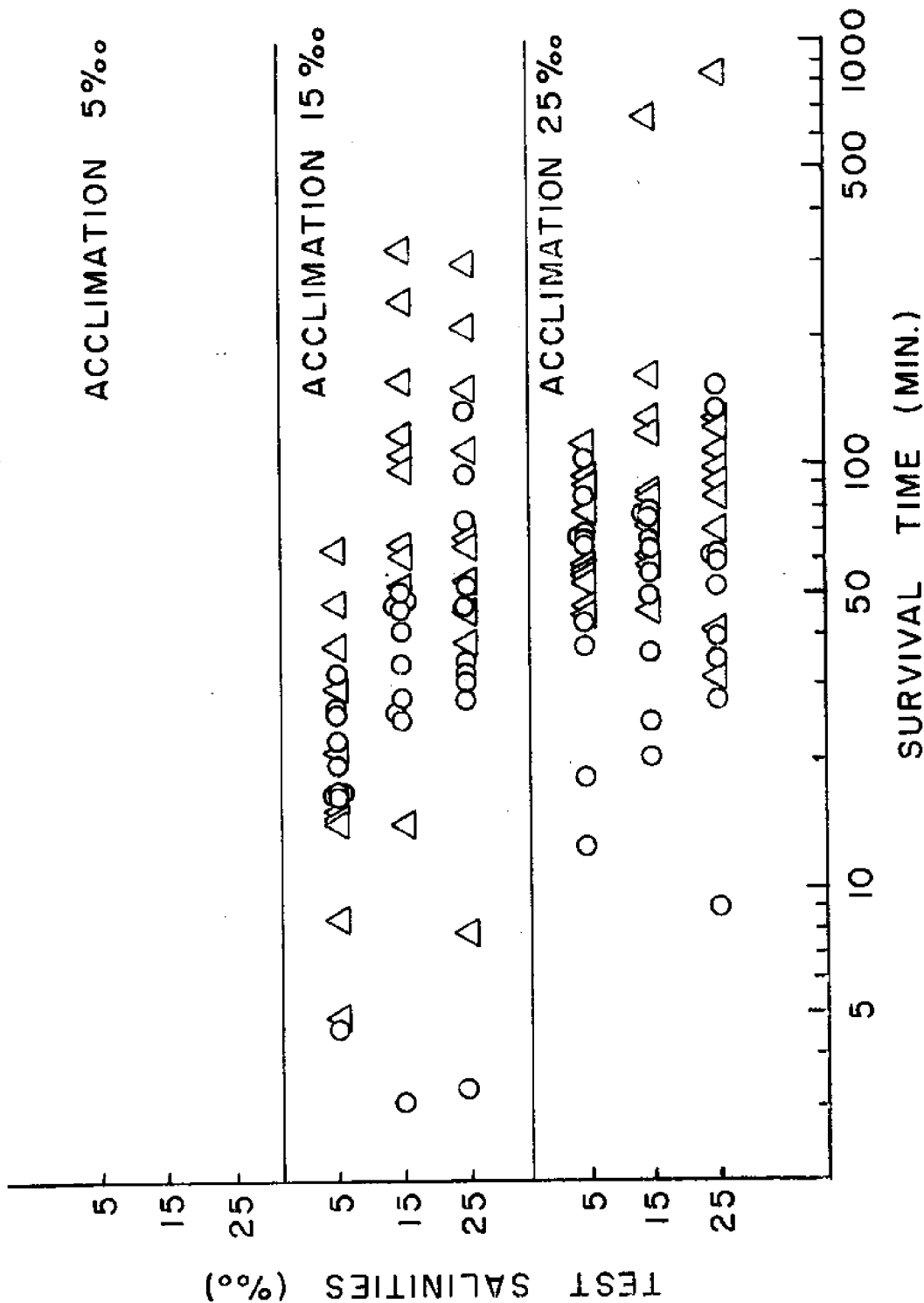


Figure 7. Survival (thermal resistance) times at different test salinities of postlarvae acclimated at 5%, 15% and 25% in the temperature salinity experiment. Acclimation temperature = 24.0°C, replication #1. High mortality of postlarvae during acclimation at 24°C and 5% precluded tests on shrimp from that temperature salinity combination for this replication. (○ = one postlarva tested at 37.0°C; △ = one postlarva tested at 36.6°C)

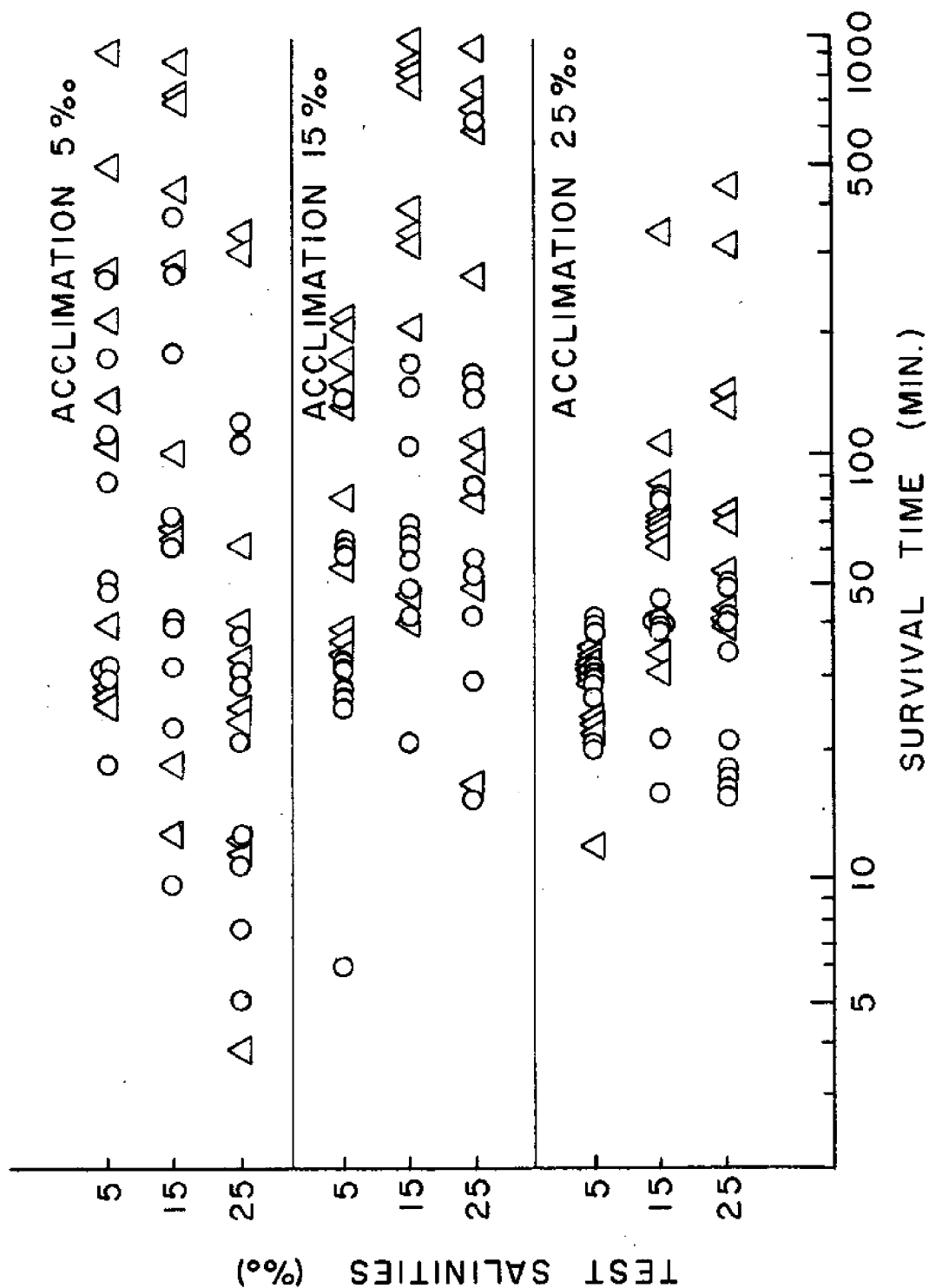


Figure 8. Survival (thermal resistance) times at different test salinities of postlarvae acclimated at 5%, 15% and 25% in the temperature salinity experiment. Acclimation temperature = 24.0°C, replication #2. (● = one postlarva tested at 37.0°C; ▲ = one postlarva tested at 36.6°C)

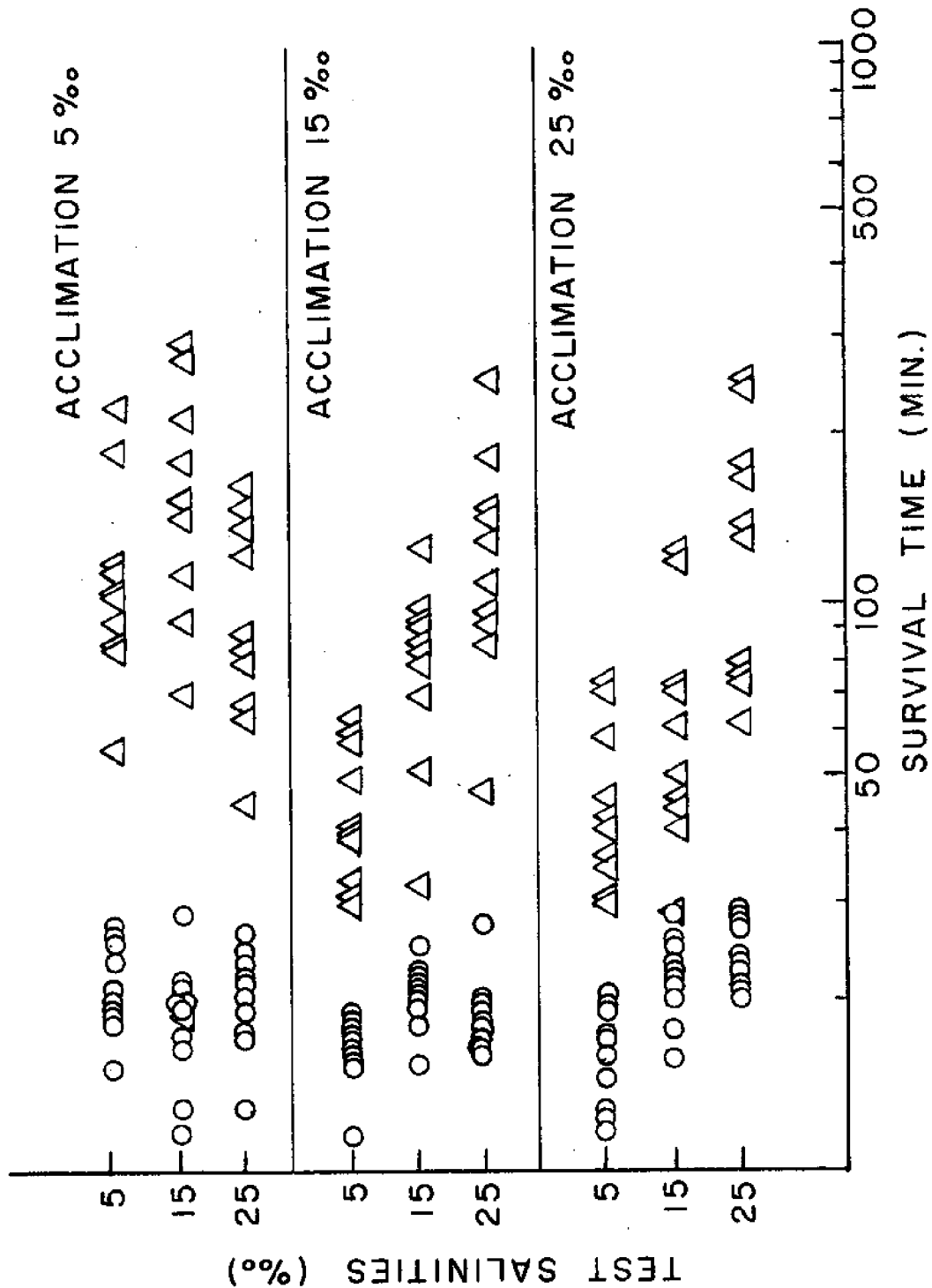


Figure 9. Survival (thermal resistance) times at different test salinities of postlarvae acclimated at 5%, 15% and 25% in the temperature salinity experiment. Acclimation temperature = 29.0°C, replication #1. (○ = one postlarva tested at 39.2°C; △ = one postlarva tested at 38.2°C)

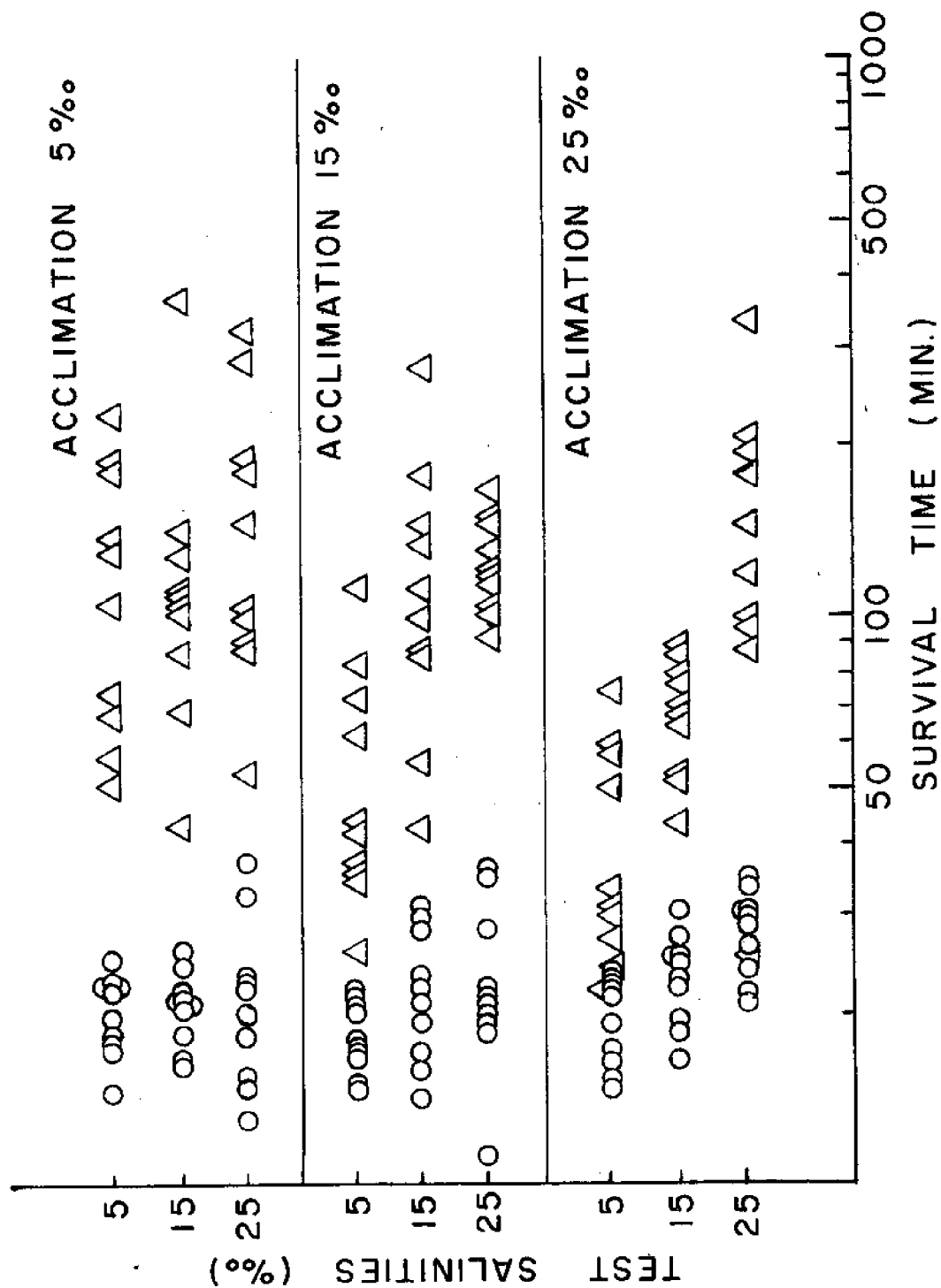


Figure 10. Survival (thermal resistance) times at different test salinities of postlarvae acclimated at 5%, 15% and 25% in the temperature-salinity experiment. Acclimation temperature = 29.0°C, replication #2.
 (○ = one postlarva tested at 39.2°C; △ = one postlarva tested at 38.2°C)

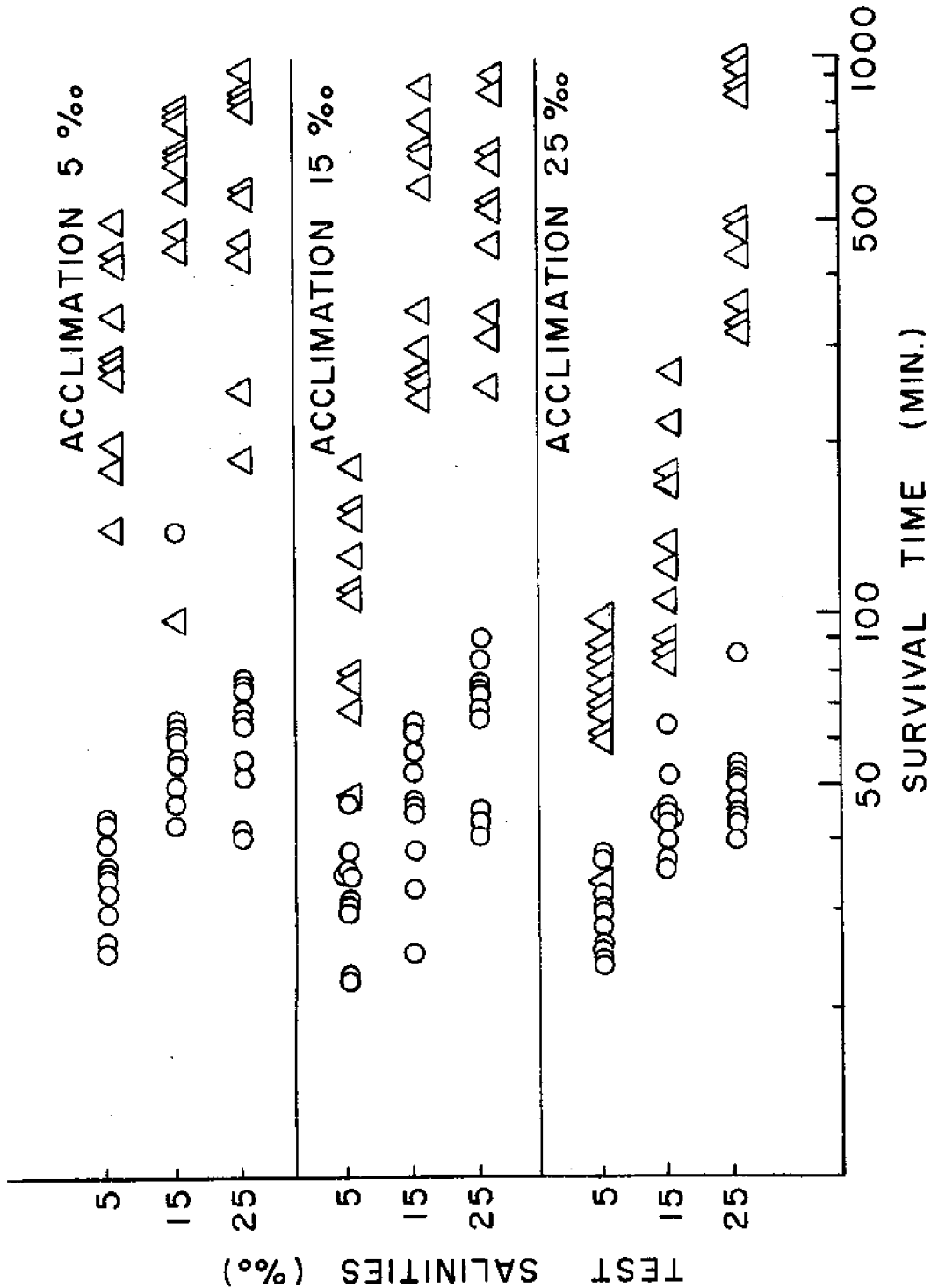


Figure 11. Survival (thermal resistance) times at different test salinities of postlarvae acclimated at 5%, 15% and 25% in the temperature salinity experiment. Acclimation temperature = 34.0°C, replication #1. (○ = one postlarva tested at 39.8°C; △ = one postlarva tested at 39.0°C)

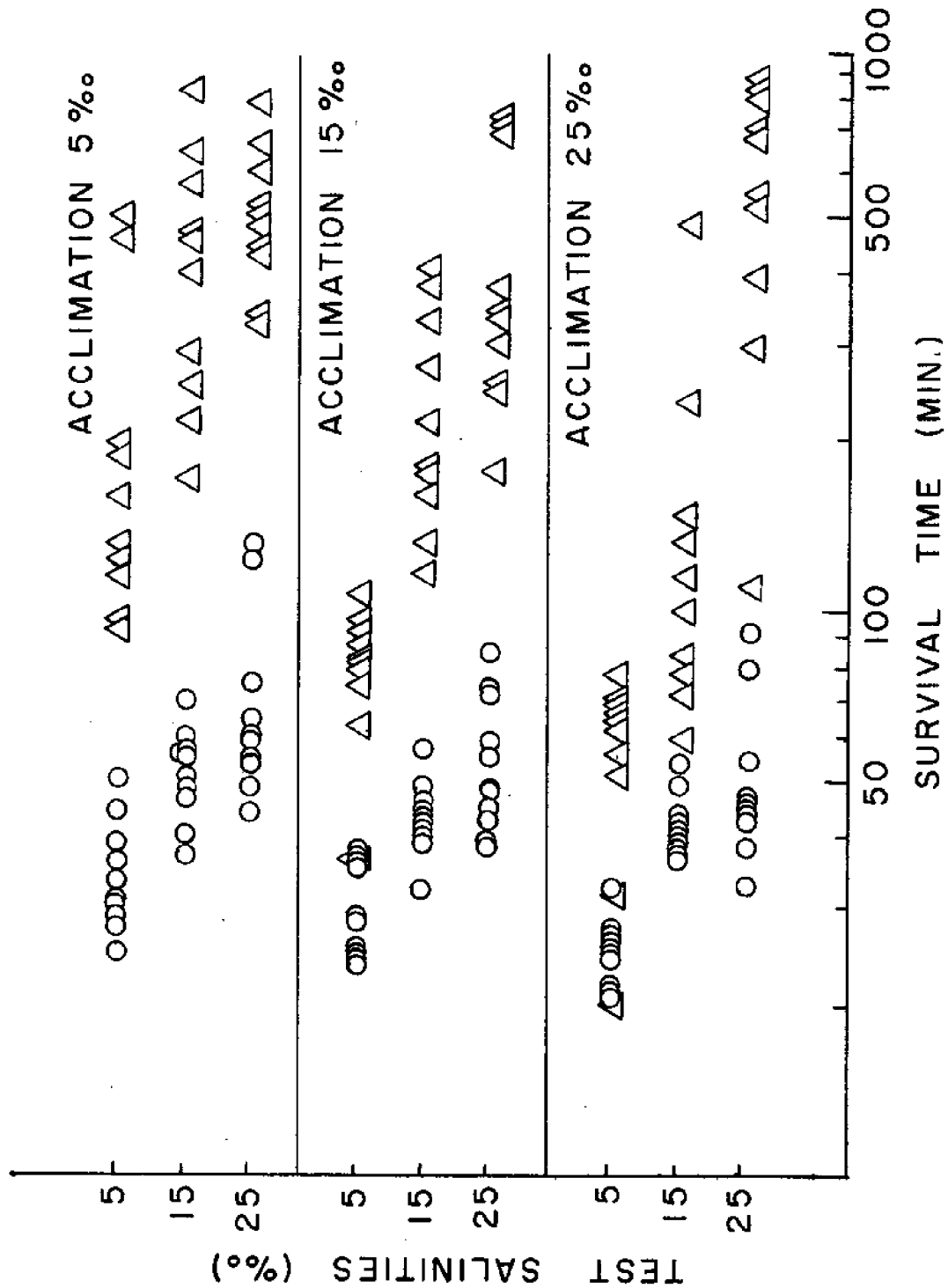


Figure 12. Survival (thermal resistance) times at different test salinities of postlarvae acclimated at 5%, 15% and 25% in the temperature salinity experiment. Acclimation temperature = 34.0°C, replication #2.

(○ = one postlarva tested at 39.8°C; △ = one postlarva tested at 39.0°C)

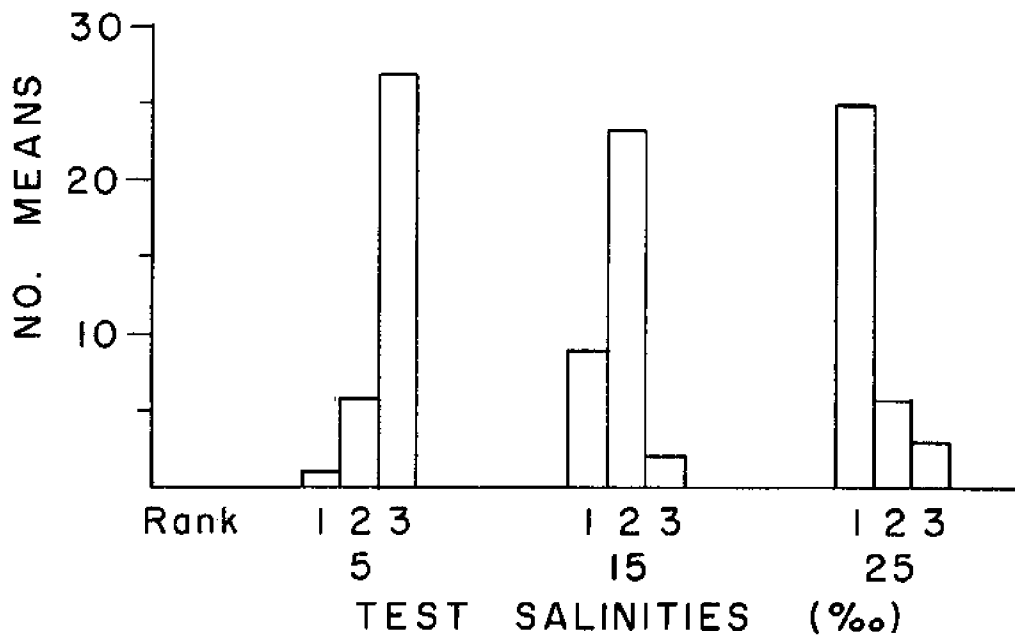


Figure 13A. (top) Frequency analysis of mean thermal resistance times of postlarvae tested at 5‰, 15‰, and 25‰ in the temperature salinity experiment. The means of the thermal resistance times of postlarvae tested at the three test salinities at each lethal temperature were ranked as 1, 2, or 3, one being the longest time and three the shortest. The number of ones, twos and threes at each test salinity were added together for the entire temperature salinity experiment. Each bar in the figure represents the number of means at each rank for each test salinity.

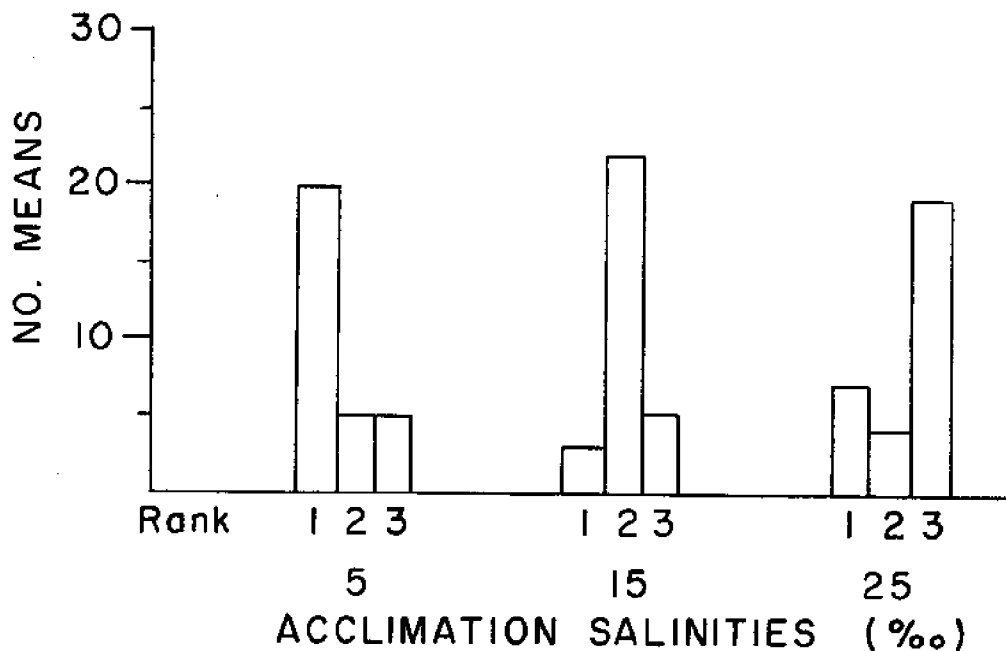


Figure 13B. (bottom) Frequency analysis of mean thermal resistance times of postlarvae acclimated at 5‰, 15‰, and 25‰ in the temperature salinity experiment. The means of the thermal resistance times of postlarvae acclimated at the three acclimation salinities at each test salinity were ranked as 1, 2, or 3, one being the longest time and three being the shortest. The number of ones, twos and threes at each acclimation salinity were added together for the entire temperature salinity experiment. Each bar in the figure represents the number of means at each rank for each acclimation salinity.

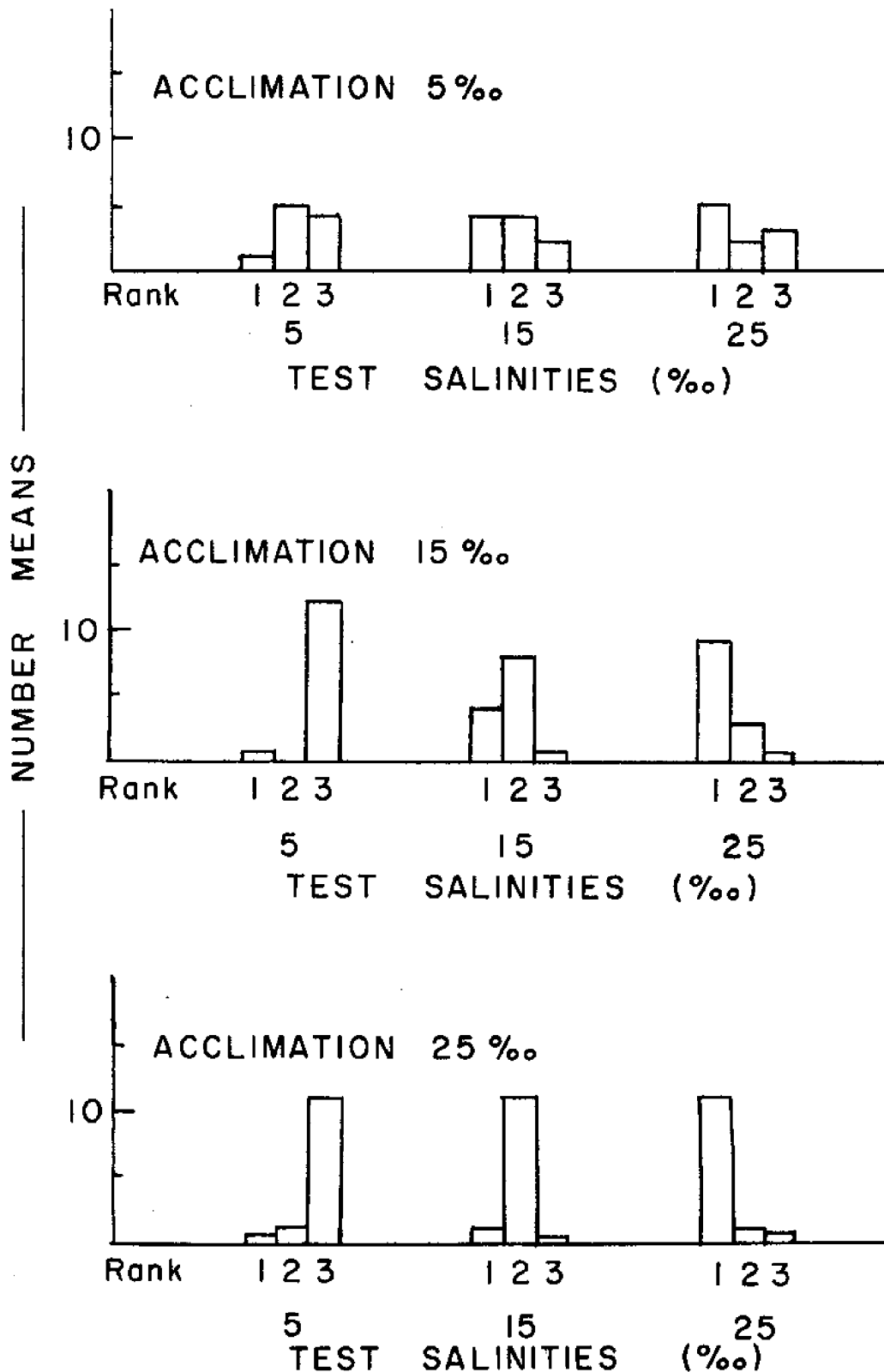


Figure 14. Frequency analysis of mean thermal resistance times of postlarvae tested at 5‰, 15‰ and 25‰ for each of the three acclimation salinities in the temperature salinity experiment. The means of the thermal resistance times of postlarvae tested at the three test salinities at each lethal temperature were ranked as 1, 2, or 3, one being the longest and three the shortest. The number of ones, twos, and threes at each test salinity for each acclimation salinity were added together. Each bar in the figure represents the number of means at each rank for each test salinity at each acclimation salinity.

NUMBER MEANS

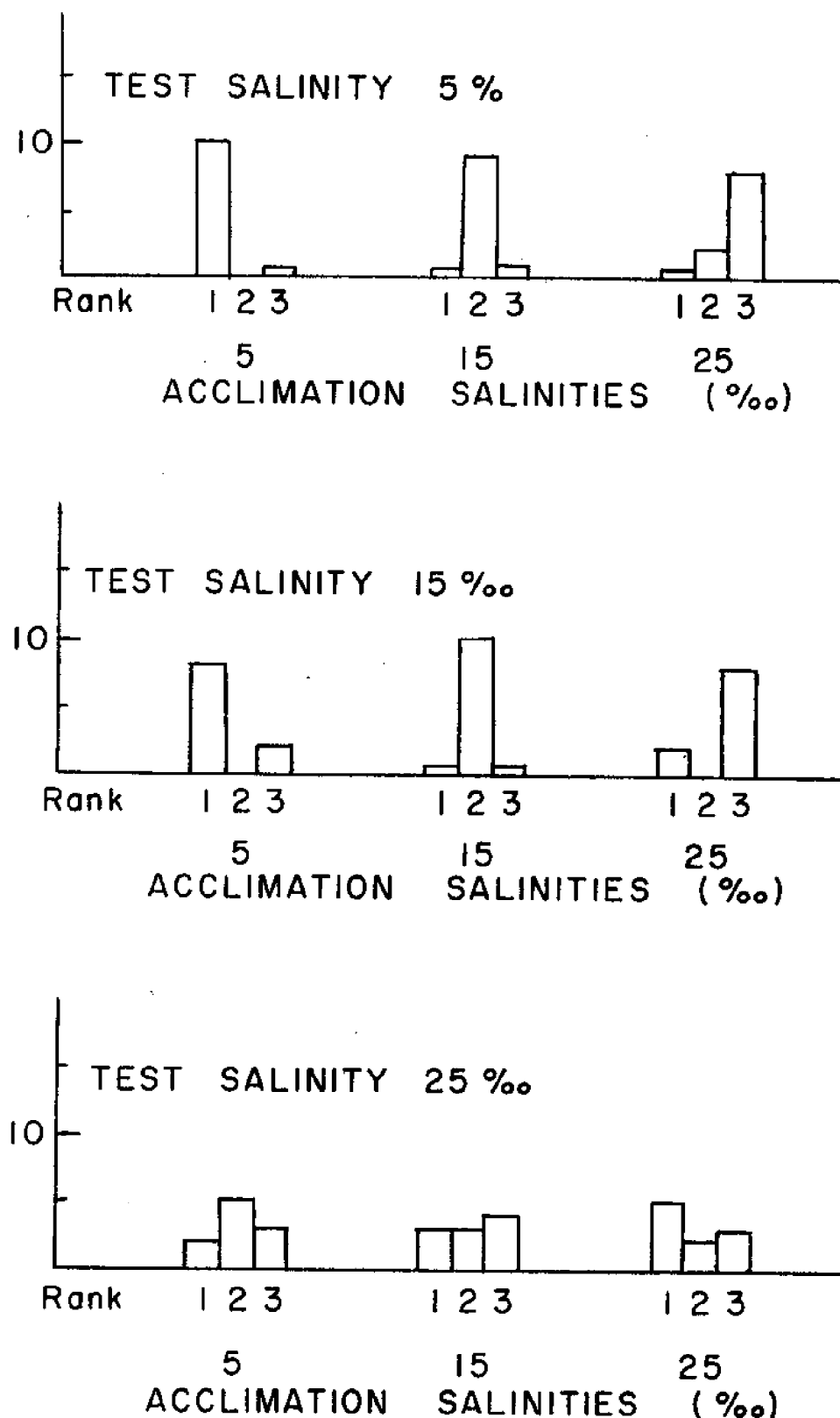


Figure 15. Frequency analysis of mean thermal resistance times of postlarvae acclimated at 5‰, 15‰ and 25‰ for each of the three test salinities in the temperature salinity experiment. The means of the thermal resistance times of postlarvae acclimated at the three acclimation salinities and tested at each test salinity were ranked as 1, 2, or 3, one being the longest and three the shortest. The number of ones, twos and threes at each acclimation salinity for each test salinity were added together. Each bar in the figure represents the number of means at each rank for each acclimation salinity at each test salinity.

For example, for postlarvae acclimated at 29.0° C and 25‰, and tested at 38.2° C (replication 1), the mean resistance time is longest for shrimp tested at 25‰ and shortest for those tested at 5‰. Thus, those tested at 25‰ were ranked as "1", those tested at 15‰ were ranked as "2", and those tested at 5‰ were ranked as "3". By combining the rankings of each test salinity for all combinations of acclimation temperature, acclimation salinity, and lethal temperature, it was possible to determine, for the entire experiment, how frequently the mean resistance time of each test salinity was longest, intermediate, or shortest of the three test salinities used. The results are plotted in Figure 20A. The other frequency analysis diagrams were prepared in a similar manner.

In Figures 16-21, isopleths are used to represent resistance times at different test and acclimation salinities. Each figure gives data for postlarvae tested at one of the six lethal temperatures. Test salinities are plotted on the "x" axis, acclimation salinities on the "y" axis. The intersects for each test salinity - acclimation salinity give nine points for which resistance times are available. These points served as guides for the construction of the isopleths.

In Figure 16, for example, the isopleths are at 10-minute intervals. The mean thermal resistance times for postlarvae tested at 5‰ were 33 minutes for those acclimated at 15‰, and 29.5 minutes

for those acclimated at 25‰. The point where the 30-minute isopleth would cross a line between the 29.5 and 33 minute intersects was determined by interpolation. The points at which other isopleths crossed lines between any adjacent pair of the nine test and acclimation salinity intersects were determined in a similar manner. The points plotted in this way were then inter-connected by lines fitted by eye.

The resulting "contour map" presents a visual picture of the resistance time at different test and acclimation salinities, the longest times being the high points. Differences between different combinations are represented by the number of isopleths between each intersect, more isopleths representing greater differences.

Statistical Analysis

The resistance time data from the temperature salinity experiment were analyzed for differences between the four main treatments (acclimation temperature, acclimation salinity, lethal temperature, and test salinity).

The statistical analysis was done on an I.B.M. System 360/65 computer, using the Estimation of Linear Models by Least Squares Program. The results of the statistical analysis are presented in Table 6.

TABLE 6
 Results of statistical analysis of data from the
 temperature salinity experiment

	Degrees of freedom	F ratio	Probability (Null hypothesis)
Replications	1	0.80107	N.S.*
Acclimation temperature	2	57.22471	<0.01
Acclimation salinity	2	6.664.4	<0.01
Lethal temperature	1	315.65221	<0.01
Test salinity	2	32.02753	<0.01

*not significant

RESULTS

Lethal Temperature Experiment

At a given lethal temperature, postlarvae acclimated at higher temperatures had longer resistance times than those acclimated at lower temperatures (Fig. 4). For shrimp acclimated at the same temperature, resistance time decreased with increasing lethal temperature.

The logs of the median resistance times for postlarvae acclimated at 29° and 34° C form a straight line, with no apparent major changes in slope over the range of lethal temperatures tested (Fig. 4). For postlarvae acclimated at 24° C, however, the slope of the line changes between 37.8° and 37.6° C.

Postlarvae acclimated at 24° C show greater variation about the mean resistance time than do those acclimated at 29° and 34° C. Shrimp acclimated at 24° C also seem to form groups of deaths at certain intervals, rather than being normally distributed around the mean (Fig. 4).

Acclimation Experiment

In both experiments, when the postlarvae did not undergo a salinity change at the same time as transfer to the higher acclimation

temperature, they gained heat tolerance at a fairly steady rate for 72 hours (Fig. 5). In the second experiment, there was no apparent gain in heat tolerance from 72 hours to the end of the experiment (168 hours) (Fig. 5). These results indicate that acclimation is substantially complete after 3 days (72 hours) in brown shrimp postlarvae.

Those postlarvae which underwent a salinity reduction from 25‰ to 15‰ (in the first experiment) exhibited no substantial difference in resistance time from those which underwent no salinity change (Fig. 6). In the second experiment, postlarvae which underwent a salinity reduction from 28‰ to 18‰ had shorter resistance times than those that experienced no salinity change, up to 96 hours after transfer. After 96 hours, there was no substantial difference between the resistance times of the two groups (Fig. 5 and 6). Apparently, it took the shrimp which underwent a salinity change 24 hours longer to complete acclimation than those which did not undergo a salinity change.

Temperature Salinity Experiment

Temperature Effects

As in the lethal temperature experiment, increasing acclimation temperature increased resistance time at a given lethal temperature (Fig. 7-12). Increasing lethal temperature decreased resistance time

for any given acclimation temperature. Statistical analysis indicated that there were significant differences between effects of different acclimation temperatures ($p < 0.01$), and between effects of different lethal temperatures ($p < 0.01$). Shrimp acclimated at 24° C (Fig. 7 and 8) showed greater variation in resistance time than those acclimated at 29° (Fig. 9 and 10) and 34° C (Fig. 11 and 12). Resistance times at all acclimation temperature - lethal temperature combinations were generally shorter than in the lethal temperature experiment.

Effects of Different Salinities Upon Resistance at a

Lethal Temperature

Generally, increasing salinity in the lethal bath (test salinity) caused increased resistance at almost all temperature-salinity acclimation combinations and lethal temperatures (Fig. 7-12). Frequency analysis shows this trend most clearly (Fig. 13A), and it is also apparent in the isopleth plots (Fig. 16-21). Statistical analysis also indicated significant differences between effects of test salinities ($p < 0.01$).

A comparison of the three acclimation salinities for each of the test salinities indicates that resistance time increased with decreasing acclimation salinity (Fig. 7-12). Frequency analysis (Fig. 13B)

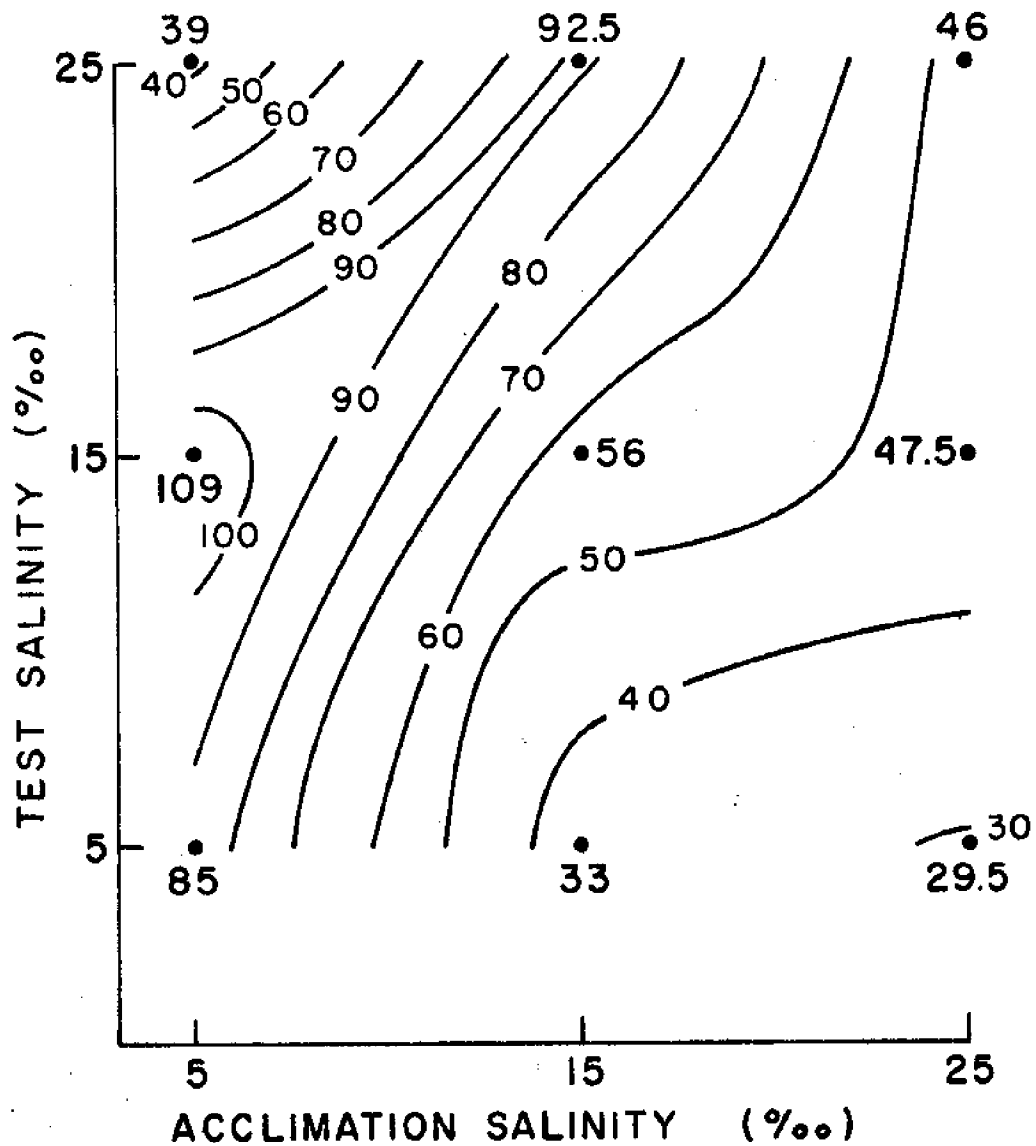


Figure 16. Contour plot of mean thermal resistance times of postlarvae at three acclimation and three test salinity levels (temperature salinity experiment). Isopleths delineate zones of equivalent death times. The isopleths were fitted by eye. Acclimation temperature = 24.0°C, lethal temperature 37.0°C.

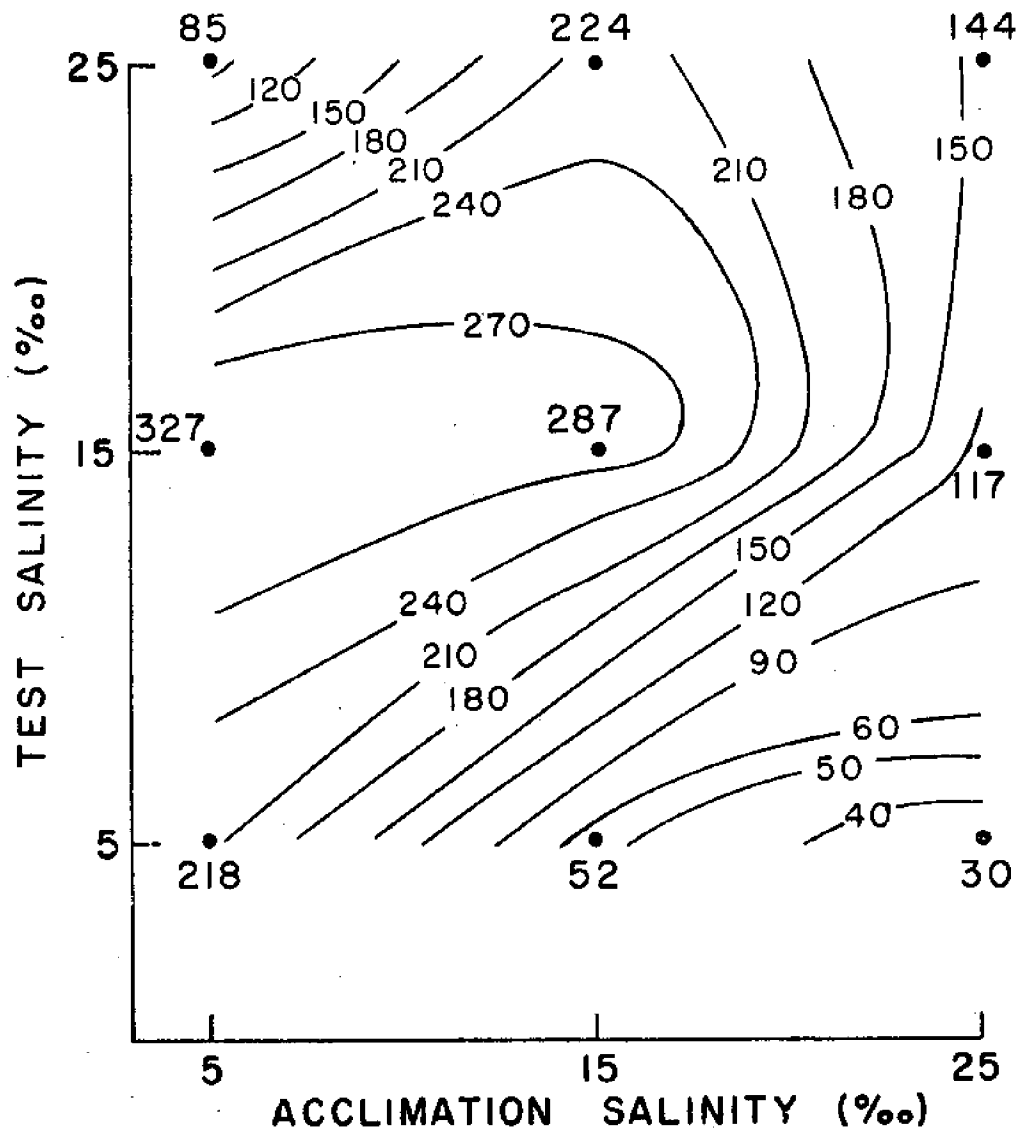


Figure 17. Contour plot of mean thermal resistance times of postlarvae at three acclimation and three test salinity levels (temperature salinity experiment). Isopleths delineate zones of equivalent death times. The isopleths were fitted by eye. Acclimation temperature = 24.0°C, lethal temperature = 36.6°C.

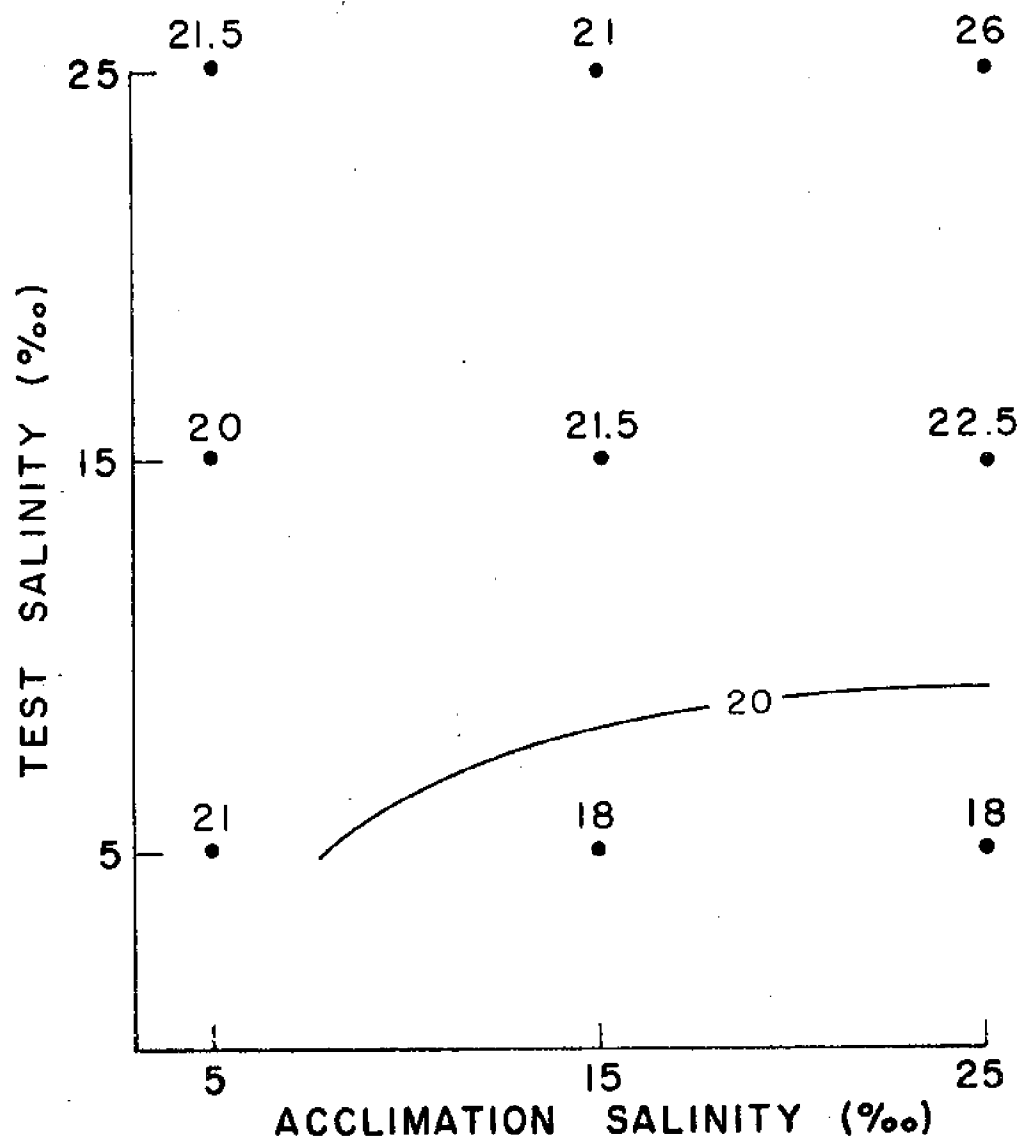


Figure 18. Contour plot of mean thermal resistance times of postlarvae at three acclimation and three test salinity levels (temperature salinity experiment). Isopleths delineate zones of equivalent death times. The isopleths were fitted by eye. Acclimation temperature = 29.0°C, lethal temperature = 39.2°C. This lethal temperature seems to have had an overpowering effect, causing resistance times at all acclimation and test salinities to be very similar.

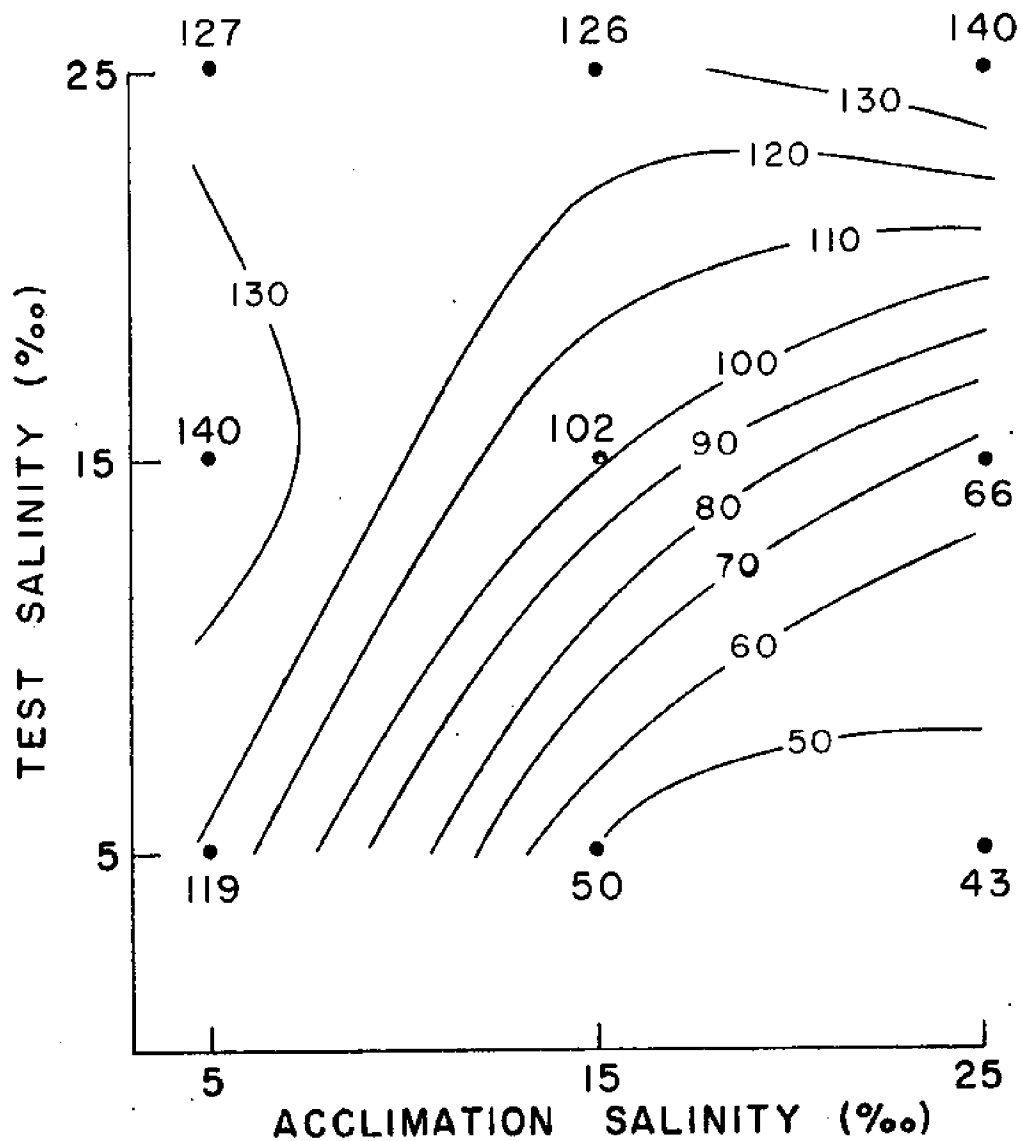


Figure 19. Contour plot of mean thermal resistance times of postlarvae at three acclimation and three test salinity levels (temperature salinity experiment). Isopleths delineate zones of equivalent death times. The isopleths were fitted by eye. Acclimation temperature = 29.0°C, lethal temperature = 38.2°C.

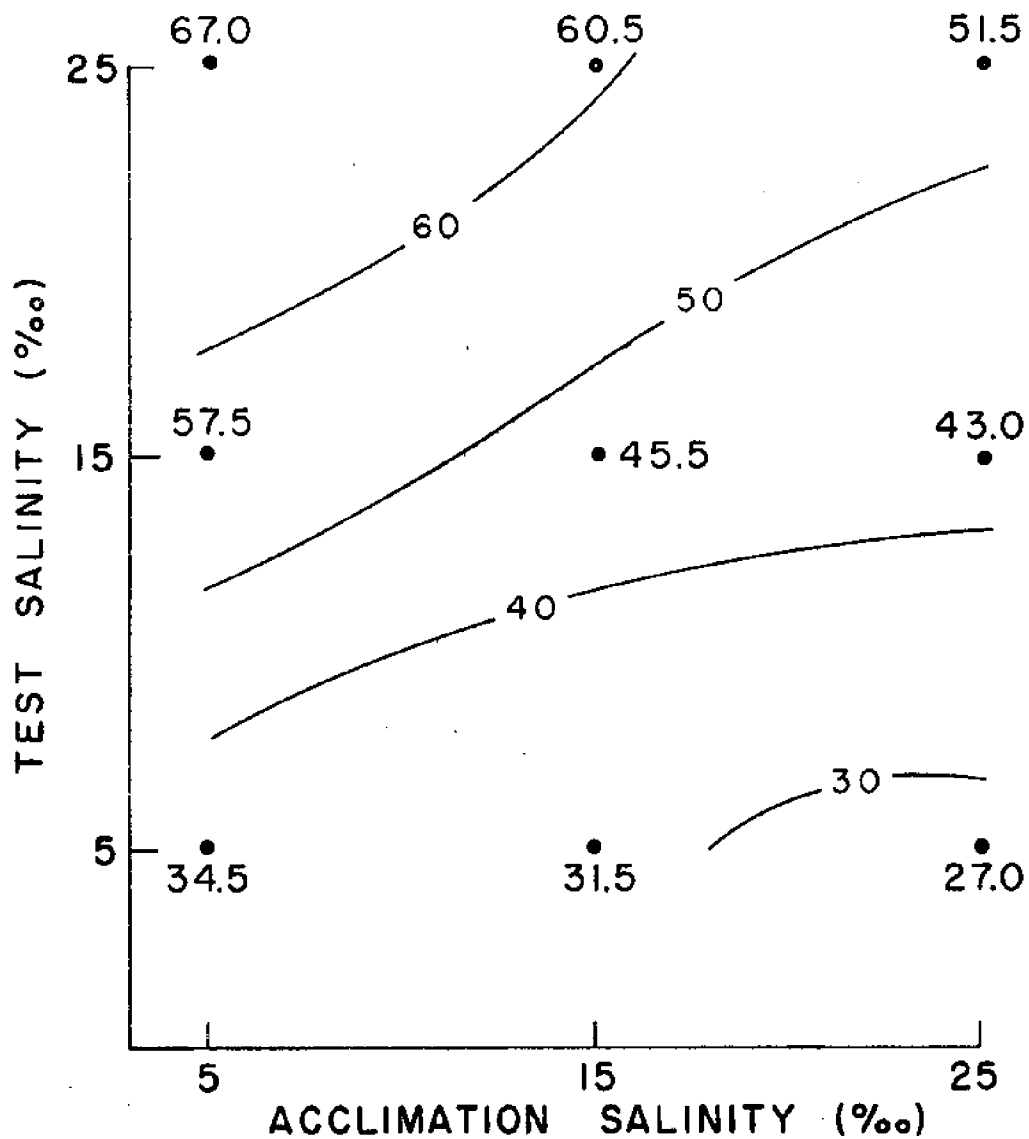


Figure 20. Contour plot of mean thermal resistance times of postlarvae at three acclimation and three test salinity levels (temperature salinity experiment). Isopleths delineate zones of equivalent death times. The isopleths were fitted by eye. Acclimation temperature = 34.0°C, lethal temperature = 39.8°C.

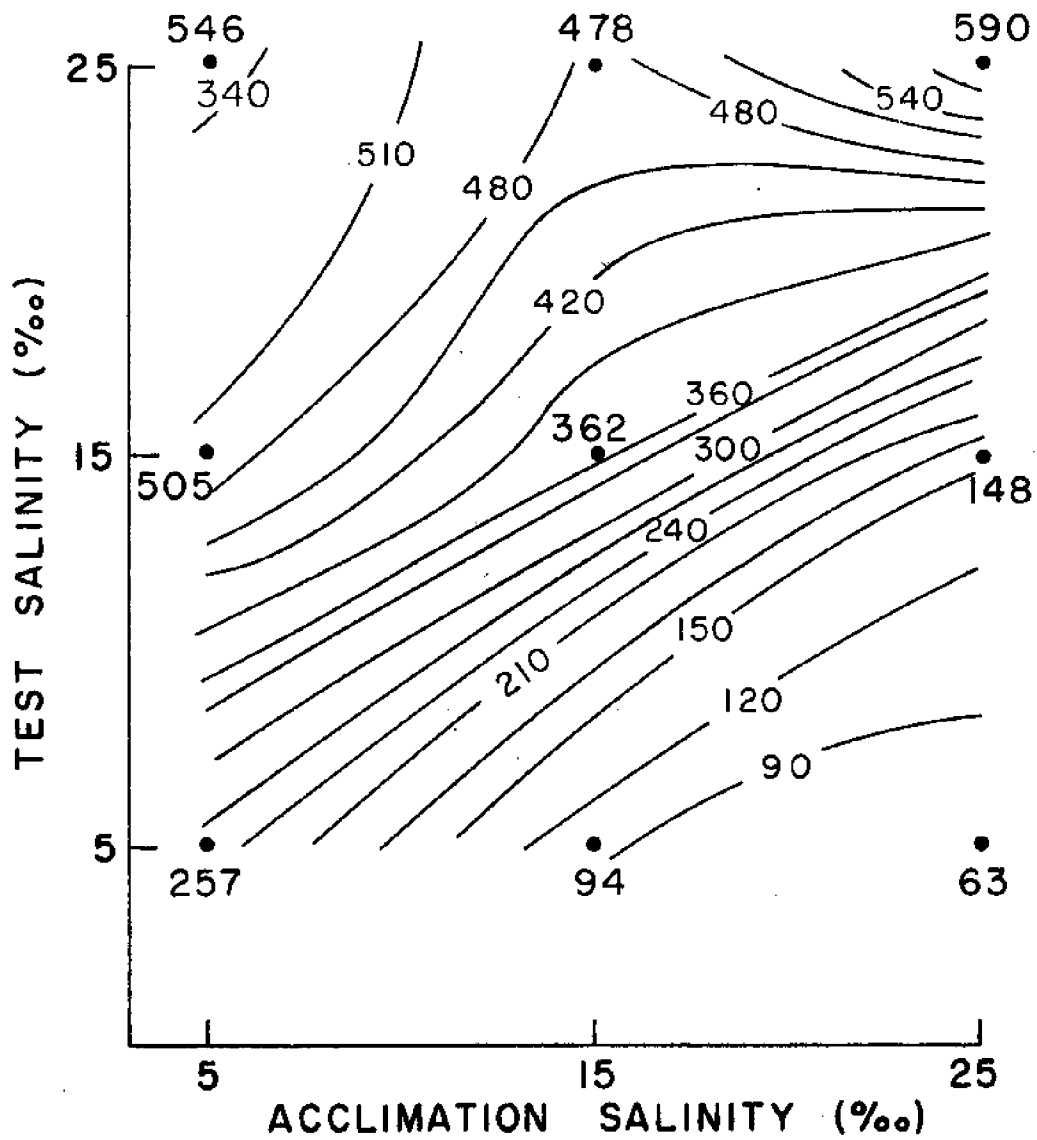


Figure 21. Contour plot of mean thermal resistance times of postlarvae at three acclimation and three test salinity levels (temperature salinity experiment). Isopleths delineate zones of equivalent death times. The isopleths were fitted by eye. Acclimation temperature = 34.0°C, lethal temperature = 39.0°C.

also indicates this trend, as do the isopleth diagrams (Fig. 16-21). Different acclimation salinity effects are also confirmed by statistical analysis, the results being significant at the 0.01 level. Thus, there appear to be two effects of salinity on resistance time in post-larval brown shrimp. When experiencing a lethal temperature, higher salinities, within the range of temperatures tested, favor increased resistance. Lower acclimation salinities, however, also lead to increased thermal resistance.

Acclimation Salinity - Test Salinity Interactions

The tendency toward longer thermal resistance times of post-larvae acclimated at 5‰ is strongest at test salinities of 5‰, while the tendency to resist high temperatures better at test salinities of 25‰ is weakest at that acclimation salinity. On the other hand, the tendency to resist high temperatures longer at test salinities of 25‰ is strongest in shrimp acclimated at that salinity, while the tendency for 5‰ acclimated postlarvae to resist longer is weakest at test salinities of 25‰. Frequency analysis of rank of mean death times for the three test salinities at each acclimation salinity (Fig. 14) and for the three acclimation salinities at each test salinity (Fig. 15) show this most clearly. This indicates that the two opposite trends produced by acclimation and test salinities affect each other to some extent.

DISCUSSION

Temperature Effects

An increase in acclimation temperature causes an increase in resistance time at a given lethal temperature in postlarval brown shrimp. This is in keeping with similar results from other studies with fishes and crustaceans.

Although the first experiment was not primarily designed to discover incipient lethal temperatures as described by Brett (1956), some information on this is available. Results of the tests on postlarvae acclimated at 24° C indicate that the incipient lethal temperature for this acclimation temperature probably lies between 36.6° and 36.8° C, as a change in slope of a line fitted to the logarithms of the median death times occurs at this point. Animals acclimated at 29° and 34° C did not exhibit any apparent change in line slope within the range of lethal temperatures at which they were tested. The incipient lethal temperatures for postlarval brown shrimp acclimated at 29° and 34° C probably are at temperatures below 38.0° and 38.6° C (or, possibly, above 39.6° and 40.2° C), respectively.

In both the lethal temperature and temperature salinity experiments, postlarvae acclimated at 24° C seemed to exhibit a greater variation in resistance time than those acclimated at the two higher

temperatures (Fig. 4, 7-12). In addition, shrimp acclimated at 24 C seem to exhibit a clumping of death times which tend to separate resistance times into more or less distinct groups.

Neill et al. (1966) and Allen and Strawn (1967) reported similar effects in two species of freshwater fishes. These authors considered the different groups to represent different physiological causes of death.

The relatively small number of animals per test in this study make differentiation of different causes of death difficult. Thermal resistance times of postlarvae acclimated at 24.0° C in the lethal temperature experiment seem to form two or more distinct groups (Fig. 4). Confirmation for this discontinuous distribution was obtained when the thermal resistance times of shrimp tested at each lethal temperature are plotted on probability paper (probability plots not shown). This has been interpreted as evidence of more than one cause of death by Fry (1957). This may indicate the presence of two or more physiological causes of death in postlarvae acclimated at 24° C. This was not the case with 29° and 34° C acclimated shrimp from the same experiment. Probability plots of data from these two acclimation temperatures showed no apparent grouping of resistance times.

Acclimation

Penaeus aztecus postlarvae seem to acclimate to a temperature increase at a fairly steady rate. The acclimation period appears to be about 3 days, after which no further acclimation occurs for a period of 4 days (Fig. 5). A comparison of the mean death times of postlarvae acclimated at 34° C and tested at 39.8° C from the lethal temperature experiment, and shrimp acclimated and tested at these temperatures in the acclimation experiment, reveal longer mean resistance times for those from the lethal temperature experiment (Fig. 4 and 5). Similar comparisons for the lethal temperature experiment and the temperature salinity experiment reveal longer resistance times for postlarvae from the lethal temperature experiment (Fig. 4, 7-12). Postlarvae from the lethal temperature experiment were acclimated for 8 days, while those in the acclimation experiment were acclimated for a maximum of 7 days, and those in the temperature salinity study were acclimated for 6 days before testing. This may indicate that the postlarvae undergo another period of increasing acclimation between 7 and 8 days after a change to a higher temperature.

The rate of gain of acclimation in P. aztecus postlarvae does not appear to be as rapid as in most other crustaceans studied

(Spoor, 1955; Bowler, 1963a; Sprague, 1963). On the other hand, this does not appear to be due to a latent period as reported by McLeese (1956) for the lobster.

Acclimation in brown shrimp postlarvae is a subject which merits further study. Tests over a period in excess of 7 days are necessary to determine if further acclimation occurs. Also, tests at different acclimation and lethal temperatures would be useful.

Salinity Effects

Generally, thermal resistance time increased with increasing salinity in the lethal bath and increased with decreasing acclimation salinities. Each of these general trends appeared to be modified by the effects of the other.

Better resistance to a lethal temperature at high than at low salinities has been reported for several other crustaceans (McLeese, 1956; Ranade, 1957; Todd and Dehnel, 1960; Tagatz, 1969). When considering resistance time as related to the salinity at which a lethal temperature is experienced, this also is generally true of post-larval brown shrimp.

Todd and Dehnel (1960) considered decreased resistance at lower salinities as due to the increased amount of work necessary for the organism to maintain the salinity differential at lower

salinities. This extra work may place an added stress on the animal, reducing its ability to resist lethal temperatures.

Adult and juvenile brown shrimp have been shown to exhibit increasing hyperosmoticity with decreasing salinities below the isosmotic salinity (Williams, 1960; McFarland and Lee, 1963). Therefore, it would appear that brown shrimp must put forth more effort to maintain their osmotic balance at 5‰ than at 25‰. Both salinity ranges used in this investigation are below the isosmotic salinity for brown shrimp reported by Williams (1960) and McFarland and Lee (1963). Assuming that postlarval brown shrimp maintain internal osmotic concentrations similar to adult and juvenile brown shrimp, the postlarvae in this study were hyperosmotic to all of the salinities used. The difference between external and internal mediums, and therefore the amount of work necessary to maintain that difference, would have been greater at 5‰ than at 25‰. Therefore, Todd and Dehnel's theory could explain the difference in resistance times at different test salinities.

When comparing postlarvae from different acclimation salinities at each test salinity, those acclimated at 5‰ usually do as well or better at lethal temperatures than animals acclimated at higher salinities. At first glance, this seems to be a contradiction of the previously discussed tendency for postlarvae to resist heat better

at higher salinities. Different acclimation and test salinities, like lethal and acclimation temperatures, may have different effects upon an organism. Thus, higher salinities are a better medium at which to resist lethal high temperatures, while lower salinities are a better preparation for heat resistance at any salinity.

An examination of the results shows that at test salinities of 5‰, the tendency for postlarvae acclimated at 5‰ to resist better than those acclimated at 15‰ and 25‰ is strongest. However, the inclination toward greater resistance at 25‰ test salinity is weakest in postlarvae acclimated at 5‰. The opposite is true for 25‰ acclimation and test salinities.

These tendencies are probably due to the opposing effects of acclimation and test salinities. In such a situation, it might be expected that one effect would be strongest at one extreme of the range of salinities tested, to the detriment of the other effect. This seems to be the case in these tests.

Mariculture Implications

It is now possible to raise brown shrimp from the egg to the postlarval stage under hatchery conditions (Cook and Murphy, 1969). Culture of Penaeus aztecus in ponds, using hatchery-produced shrimp now seems feasible.

Cook and Murphy (1969) have found that the range of salinities at which brown shrimp will hatch and develop is relatively narrow (27‰-35‰). Hatchery shrimp reaching the postlarval stage would be at fairly high salinities. The results of this investigation indicate that acclimating the hatchery postlarvae to lower salinities before transfer to rearing ponds would better prepare the young shrimp for the changes in temperature and salinity they would face. However, the stage at which the postlarvae acquire the ability to withstand lowered salinities must be determined first. If young shrimp are held in out-door ponds during the summer, low salinity would be the best preparation for meeting high temperatures.

Ecological Implications

Gunter (1950, 1961) has theorized that young white and brown shrimp need low salinities as a condition for survival. This theory is based on field observations which indicate decreasing size of shrimp with decreasing salinity (Gunter, 1950), and a reported correlation between high rainfall (causing lowered salinities in the nursery areas) and good commercial catches of white shrimp (Gunter and Hildebrand, 1954). Gunter et al. (1964) stated that young brown shrimp are most abundant in the salinity range 10‰-30‰, with most

found at 10‰-20‰. Most of these studies are based on data on juvenile shrimp, although postlarvae are thought to be similarly affected.

A need for low salinity by postlarval brown shrimp has been questioned by Zein-Eldin (1963) and Zein-Eldin and Aldrich (1965). In laboratory studies, they found that salinity by itself had little effect on growth and survival of brown shrimp postlarvae. Survival at low temperatures (below 15° C) was adversely affected by low salinity, however (Zein-Eldin and Aldrich, 1965).

Data on temperatures in shrimp nursery areas in upper Trinity Bay, Texas (Truesdale, 1970) indicate that temperatures do not approach the lethal levels found in this study in the spring, when brown shrimp postlarvae are in the shallow parts of the bay. Studies by Aldrich (unpublished data) indicate that temperatures in the shallow water immediately next to the shore are often higher than waters a short distance offshore. Temperatures in the range of lethal temperatures found in this study (above 38° C) were sometimes recorded in mid-summer. Mock (1966) found that most brown shrimp postlarvae were located in these shallow near-shore areas. It is possible, however, that shrimp exposed to these high temperatures would have had the opportunity to become acclimated to fairly high temperatures, and would be able to resist the lethal effects for some

time. It seems likely that brown shrimp postlarvae are seldom exposed to rapidly lethal high temperatures under natural conditions.

It is well known that most organisms have upper lethal temperature limits well above the highest temperature that they normally experience. This provides a safety factor which insures that the animal will not have to constantly face detrimental high temperatures.

For brown shrimp postlarvae, acclimation at 25‰ provides adequate thermal resistance at that salinity. Resistance is substantially reduced when the animal is faced with 5‰. On the other hand, acclimation at 5‰ provides for near-optimum thermal resistance at 5‰, as well as at higher salinities up to 25‰.

In the changing environment of the bays, postlarvae which seek out and live at 5‰ would have the greatest protection against adverse high temperatures no matter what the salinity. Shrimp living at higher salinities would experience a reduction of thermal resistance if the salinity were reduced. Therefore, living at 5‰ would insure an adequate "buffer zone" against lethal temperatures, no matter what the salinity.

This appears to be an adaptation for coping with the constantly changing environment of the bays. It does not explain why the postlarvae leave the sea, where stability of salinity would preclude

the need for such an adaptation. The reason that postlarvae enter the bays is not known. It may be food requirements, as suggested by Zein-Eldin (1963). Reduced competition and/or predation is another possible benefit of entering the unstable environment of the bays, if competitors and predators cannot tolerate these conditions (Kinne, 1967). Near-optimum thermal resistance at all higher salinities for postlarvae acclimated at low salinities, however, is probably an adaptation which allows the postlarvae to enter the bays, rather than the reason that they do.

While lower salinities provide postlarval brown shrimp with an excellent preparation for resisting extreme high temperatures, higher salinities provide a better medium at which to experience high temperatures. This would seem to indicate that it would be profitable for the postlarvae to seek more saline waters when experiencing high temperatures.

Kinne (1967) has suggested that when faced with an extreme of one environmental factor, an organism might reduce the detrimental effects by seeking the optimum condition of a second factor, which interrelated with the first, to reduce the effects of the first factor. He termed this "indirect escape".

Mock (1966) reported, in a study of postlarval shrimp distribution, that salinities in the shallow areas next to shore were very

similar to those 100 yards offshore. Any search for higher salinities in a horizontal direction might involve an extensive migration for postlarvae. Another possible method of indirect escape is burrowing in the substrate, as waters within the substrate are sometimes more saline than the waters above it (Siever et al., 1961). Penaeus aztecus postlarvae have been shown to burrow in response to low temperatures (below 17° C) (Aldrich et al., 1968). It is possible that high temperature extremes could cause a similar response.

The effects of thermal pollution on postlarval brown shrimp would depend primarily on the temperature of the discharge water and the previous thermal history of the animal. The results of this study indicate that salinity is also a factor that must be considered. The salinity of the discharge water, as well as that of the surrounding water, could have an effect on how well postlarval brown shrimp could tolerate heated waters.

The effects of different acclimation salinities on thermal tolerances at different test salinities has apparently not been established for other marine animals. Most other temperature salinity studies have tested the organism being studied at the same salinity as the acclimation salinity. It would be of interest to determine whether the response to environmental salinity described here for P. aztecus postlarvae is a characteristic of other euryhaline animals.

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