SALINITY AND UPPER TEMPERATURE TOLERANCES OF A ROCKPOOL COPEPOD, *TIGRIOPUS CALIFORNICUS* (BAKER, 1912)

by

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INTRODUCTION

The most fundamental biological response domain is that of tolerance which defines the limits within which survival is possible. Within the maximum tolerance range are the lesser domains of other biological qualities such as locomotion, growth, and reproduction, these commonly varying according to stage of development. Determining these limits becomes equivalent to delineating the potential distribution of a species.

Carl Schlieper has said, "No aquatic organism can evade the influence of its surroundings" (Remane and Schlieper, 1971). Even though marine and brackish water organisms subject to a variety of environmental factors must respond to the total stimulus or stress rather than any one environmental parameter, temperature and salinity represent two of the most potent physical factors. Marine temperatures may vary between -2 and 30° C. while neritic waters commonly range from -3 to 43° C. Though relatively narrow when compared to terrestrial values certain aquatic situations can experience temperature fluctuations of nearby terrestrial environments. In the open ocean salinities range from 33 to 37 o/oo though the Red Sea with 40 o/oo and the Baltic with values as low as 2 o/oo represent extremes. There do exist bodies of water like the Laguna Madre in Texas where 50-80 o/oo are common and shore ponds in the tropics may reach values of 155 o/oo (Kinne, 1964). In nature the total salinity range from zero to full saturation at 260-280 o/oo may be found. When dealing with an organism and an environment it may be far more important to deal with the range and pattern of salinity and temperature fluctuation than average values.

It becomes increasingly obvious that temperature and salinity should be considered together as environmental parameters because each is capable of influencing the effects of the other. Additional biotic or abiotic factors may exert similar influences. When ecological implications are drawn from univariable laboratory experiments there exists doubt as to their validity when confronted with the multidimensional nature of the real environment. Contrarily, if approaching a question from a field perspective the complex nature of parameters acting on the issue in question may create confusion in trying to separate out or detect their influences. A compromise of sorts is realized by laboratory experiments that seek to examine several parameters acting in concert. Difficulties in analysis and design pose problems, but the conclusions arrived at may prove to be more realistic regarding the responses of organisms to their environments.

In spite of the important physiological and ecological consequences of the combined effects of temperature and salinity on living systems the information at hand is rather limited. Previous work on the temperature and salinity effects on marine invertebrates has been reviewed by Kinne (1963, 1964, 1967, 1970). Other general treatments can be found in Remane and Schlieper (1971), Vernberg and Vernberg (1972a), and Vernberg (1975). Specific studies on the joint effects of temperature and salinity on marine invertebrates have been carried out on the lobster, *Homarus americanus* (McLeese, 1956), the blue crab, *Callinectes sapidus* (Costlow and Bookhout, 1959; Costlow, 1967), *Hemigrapsus nudus* and *H. oregonensis* (Todd and Dehnel, 1960; Dehnel, 1960; Hulbert, *et al.*, 1976), the grass shrimp, *Palaemonetes* sp. (Thorpe and Ross, 1975), larvae of the coot clam, *Mulinia lateralis* (Calabrese, 1969), the mud crab, *Panopeus* (Christiansen and Costlow, 1975), and several Baltic rockpool ostracods (Ganning, 1967, 1971b). Investigations to the extent which free living copepods can endure extremes in salinity and temperature are limited, and often the factors are not correlated. Experiments have been conducted with *Calanus finmarchicus* (Marshall and Orr, 1972) and

Eurytemora affinis (Bradley, 1975). The salinity resistance of a few oceanic forms has been studied by Hopper (1960) and estuarine forms by Lance (1963) and Moriera (1975). Temperature tolerance work has been done with various species of *Tigriopus* (Ranade, 1957; Matutani, 1961; Kontogiannis, 1971). Clark (1968) and Vittor (1971) have investigated some effects of joint salinity-temperature interaction on *Tigriopus*. All these studies have pointed out facets of temperature and/or salinity effects on the copepods involved but few have offered any conclusions regarding the effects of a wide range of salinities on thermal tolerance.

Tigriopus californicus as an inhabitant of supralittoral rockpools is subject to sudden and violent fluctuations in salinity and temperature. These conditions have eliminated this niche for most marine invertebrates (Ricketts and Calvin, 1973). *Tigriopus* must possess remarkable tolerances to be able to successfully withstand the rigors of its environment. Accordingly it would be a convenient animal for assessing the joint effects of a wide range of salinities, acclimation temperatures, and elevated stress temperatures on mortality. The experiments described here were designed to characterize the salinity and upper temperature tolerances of *Tigriopus californicus* as a representative organism of the rockpool environment.

THE GENUS AND THE SPECIES

Marine harpacticoid copepods comprise a very diverse and extensive group being particularly common in the littoral zone. The genus *Tigriopus* Norman has a world-wide distribution. It is often the dominant species in supra-littoral rockpools. *T. californicus* (Baker, 1912) is the form found in the eastern Pacific from Baja California (Vittor, 1971) to Sitka, Alaska (personal observation). *T. triangulus* has been shown to be the same as *T. californicus* (Smith and Carlton, 1975). The species *T. japonicus* is found on western Pacific shores (Matutani, 1961). *T. fulvus* was originally thought to encompass all European forms but in 1960 Božić (cited by Comita and Comita, 1966) demonstrated genetic and morphological grounds for dividing European specimens into two distinct species: the southern form, *T. fulvus*, of the Mediterranean and warmer waters and *T. brevicornis* of Scandinavia and the British Isles. Species differ only slightly in morphology (Monk, 1941) and size leading Shaw (1938) to describe his California collected animals as *fulvus*. Fraser (1936 a, b) and Ranade (1957) called their British forms *fulvus*.

A typical harpacticoid copepod, *Tigriopus* is equipped with mouth parts for biting, gnawing, and chewing. It is not thought to filter feed. It utilizes epiphytic organisms and possibly some portion of the common rockpool algae, Enteromorpha intestinalis, but the majority of its food is provided by micro-organisms (bacteria, protozoans, rotifers, etc.) associated with bottom deposits (Clark, 1968). Feeding on juvenile forms may occur incidental to detritus, though cannibalism of recently deceased copepods has been occasionally observed.

The bright orange color of recently collected animals may be due to the incorporation of carotenoid pigments from ingested plant material (Clark, 1968; Vittor, 1971). Fraser (1936a) attributes the color to the chitinous exoskeleton. Both undoubtedly are involved since laboratory maintained animals become pale orange when plant material is not available. It is not known if the coloration has any particular significance to the animal.

The sexes are distinct in adult stages. Males possess easily identifiable geniculate first antennae used in grasping the female during copulation. Females have finer antennae and the common presence of a single pink egg sac makes them distinct from males. During mating the adult male swims above the adult female or fifth stage female copepodid attached by the hooks of the first antennae. The amplectic pair may spend several hours to several days together during which time a spermatophore is transferred to the female. This will suffice for the development of up to twenty broods of eggs (Vittor, 1971). Five to ten days after copulation, depending on temperature, or soon after the final moult if the female had mated at the fifth copepodid stage, a small dark egg sac appears behind the fifth leg of the female. It grows quickly changing to pale green and eventually assuming a bright pink color. The eggs (average 46, range 10-110 [Vittor, 1971]) remain in the sac from ten to fifteen days and then hatch while the sac is still attached to the female. The newly hatched animal is a first nauplius. The naupliar and metanaupliar stages have a duration of about eight days; the subsequent copepodid larval stages, nine to twelve days (Shaw, 1938). There is lack of agreement in the literature as to how many distinct naupliar and copepodid stages exist for Tigriopus californicus. Shaw (1938) reports five naupliar and four copepodid; Huizinga (1971) four and four; Vittor (1971) six and six; Matutani (1961) describes T. japonicus as having six and five; and Fraser (1936a) describes five naupliar and five copepodid stages for T. brevicornis. Regardless, the final copepodid moults into the adult stage and no further moulting takes place.

Adult males (~1.1 mm.) are generally smaller than adult females (~ 1.3 mm.). The average time from egg to reproductive adult is temperature dependent being approximately 32 days at 15° C. (Comita and Comita, 1966). Longevity varies similarly being about 129 days at 15° C. (Vittor, 1971).

Lack of predators in high rockpools combined with rapid generation time permits Tigriopus to achieve densities as high as 8900 per liter (Ranade, 1957) or undergo changes in population increase from ten gravid females to several thousand adults in a month's time (Vittor, 1971). Rarely is food a limiting factor (Clark, 1968).

The question of dispersal of the animal and colonization of pools has not been determined. Towing plankton nets just off rocky shores failed to produce Tigriopus (Vittor, 1971). The amphipod Gammarus duebeni is similarly not found in the sea but has a euryhaline distribution in rockpools (Hynes, 1954). It seems probable that colonization and dispersion are accomplished by inshore current transport of animals from pools which are essentially permanently populated. Once installed in a pool the animal's reproductive capacity and high temperature and salinity tolerance provide them with a rather exclusive niche.

THE ROCKPOOL ENVIRONMENT

Along rocky coastlines the gathering of water in various depressions gives rise to a special ecological niche. Direct wave or tidal action will fill some pools while others will be influenced only indirectly by the sea through spray or salt carried by rain. The lowest rockpools have conditions approaching the nearby marine environment, but as one moves high into the littoral zone or beyond conditions become much more extreme. Various schemes have been proposed to classify rockpools (reviewed by Clark, 1968), but the difficulty with any classification system is that clear-cut differences between types may change with time or that a continuity of types exist that makes categorizing not clearly possible. Ganning (1971) has classified rockpools into five general types:

I. Permanent salt-water pools: pools of various sizes that receive sea water intermittently by tidal or wave action; reasonably stable conditions.

II. Ephemeral salt-water pools: pools of samller size and usually higher up on the shore than Type I, being filled primarily by splash or heavy spray; accordingly conditions are subject to great variations.

III. Brackish water pools: pools usually high on the shore more influenced by rain than the sea, hence, lower salinities; also subject to extremes.

IV. Permanent freshwater pools: primarily limnetic in nature, they are situated so as to receive only occasional sea spray; depending on volume and water source (rain, runoff, seeps, springs) they can provide a stable set of conditions.

V. Ephemeral freshwater pools: being of small volume but of a character similar to Type IV these pools are inclined to dry up in warm weather and provide a variable, temporary habitat.

The first three types are of primary importance to organisms with immediate marine associations while the latter two being limnetic in character have a less related fauna and flora. The rockpools of the upper and supralittoral are more influenced by ambient conditions than any other marine situation. Violent fluctuations in physical factors makes this an extreme environment in the sense that severe limitations are imposed as to which species can successfully live there (Brock, 1969). It is an environment characterized by low species diversity though not necessarily low individual numbers.

In their natural environment organisms are always being affected by many factors simultaneously. The interplay among these parameters is usually of more importance than single isolated ones (Alderdice, 1972). When speaking of the physical and/or biological components of a situation it is easy to examine them piecemeal forgetting that they need be related wholistically. Thus, even though salinity, temperature, oxygen, photoperiod, predation, etc. are conveniently studied and talked about as entities it is important to consider them as impinging components in a larger picture. Rockpools with so many fluctuating parameters are in some ways convenient natural aquaria in which to study individual factors and combine them into a synthesized overview.

Among the most important factors in rockpools is temperature. It is influenced in a number of ways. There will be seasonal and latitudinal differences. Local weather, time of day, and exposure will determine heat gain or loss. The addition of water in the rockpool by rain or seawater will modify it. The presence of a freshwater lens due to rain or through-flow on top of denser saline water may occasionally produce a "greenhouse effect" to intensify solar heating. Basically small pools are more affected than larger ones. For example, small exposed pools on the Oregon coast may experience diurnal differences as much as 15° C. whereas large ones only a few degrees in the same time period. Annual temperature variation in Oregon coast pools would extend several degrees on either side of the 4-28° C. range reported by Vittor (1971) in Cape Arago pools. While clearly paralleling local climatic conditions modifying factors such as the "greenhouse effect" mentioned above will raise temperatures above ambient air. Clark (1968) states that for pools she studied in Scotland water temperatures followed air temperatures for most of the year except in the May through September period when the pools averaged higher than air. Compared with the sea the pools were usually warmer in summer and cooler in winter unless recently inundated. Thermal stratification may develop especially in protected pools, but the usual large surface to volume ratio of rockpools allows thorough mixing by the wind.

Klugh (1924) and Stephenson, et al. (1934) are among the authors that point to temperature as a prime limiting factor for organisms in rockpools. It should be noted that the pools they investigated were intertidal and not subject to the variety of factor fluctuations found in smaller supra-littoral pools. The rhythmic cycle of tides adds a stabilizing effect to damp violent fluctuations in lower pools. Nonetheless temperature is important in determining tolerance ranges and zones of metabolic, reproductive, and developmental optima. For example, T. californicus achieves reproductive maturity in 32 days at 15° C. but at 20° C. needs 27 days and 25° C. only 18 days. Longevity is also influenced: 129 days at 15° C., 116 days at 20° C., and 85 days at 25° C. (Vittor, 1971).

Salinity is commonly regarded as the dominant factor affecting the organization of rockpool communities and their maintenance. As mentioned above it is a useful basis for classification. The influence of climate and shore morphology vary the effects of evaporation, precipitation, runoff, and input of sea water or spray and determine the extent and duration of salinity conditions. Large deep protected pools will be more stable than small shallow exposed ones. High supra-littoral pools will have variable salinity if they receive appreciable input from the sea or spray. Sub-saline pools that receive salt primarily via precipitation or runoff will show much less change in salinity relative to evaporation. On the Oregon coast summer and fall are the seasons when evaporation increased by wind will produce the greatest influence. Precipitation as rain or snow can offset the effects of evaporation during this period but more commonly has its influence in the winter and spring. Freezing of pools will produce increased salinity and severe storms, heavy rains, and high tides can cause swift changes. On the Oregon coast the common range of salinities in rockpools is 10-45 o/oo, but pools between 0 o/oo and 80 o/oo may be encountered depending on the season (Vittor, 1971; personal observation). Obviously a pool evaporating completely will have values approaching full saturation at 260-280 o/oo. Salinity stratification is unusual except in the case of freshwater layering on the surface. Horizontal gradients are possible in saline pools after precipitation and are caused by freshwater flooding, mixing, and through-flow.

An important but often neglected aspect of rockpools is the fact that the ionic composition of the saline water may differ appreciably from that of the sea. Ganning and Wulf (1969) noted the influence of increased nitrogen-ammonia and phosphorus-phosphate from bird droppings on variations in physical factors and faunal composition. Runoff from the land will carry other ions usually associated with fresh water, e.g., magnesium and bicarbonate (Järnefelt, 1940 as cited by Ganning, 1971a). In pools subject to complete evaporation differential precipitation may occur altering ionic composition.

Salinity will exert its effects on the ecology of rockpools by acting in concert with other physical parameters, i.e., temperature, oxygen, pH. Still as shown by classification schemes and discussions

(Pyefinch, 1943; Naylor and Slinn, 1958; Clark, 1968) salinity is a useful focus in examining this environment. As such the ecology of various ostracods from Baltic brackish water rockpools has been studied (Ganning, 1971b). Environmental partitioning on the basis of salinity optima in relation to survival and development are examined. When offered a salinity choice *Gammarus duebeni* and *Heterocypris salinus* showed preference for waters from which they were taken as opposed to higher or lower salinities (Ganning, 1967).

As would be expected most successful inhabitants of rockpools exhibit a large measure of euryhalinity. Such tolerance investigations are among the most common experimentation associated with rockpool animals and will be examined in more detail later. A few examples will illustrate salinity tolerances. *T. brevicornis* was capable of surviving normally in a range of 4.2-90.0 o/oo (Ranade, 1957). The common european amphipod *Gammarus duebeni* can successfully tolerate a range between 0 and 80 o/oo (Kinne, 1964). The crabs *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* thrive in pools at 66 o/oo (Vernberg and Vernberg, 1972).

Within the tolerance zone, salinity exerts influences on metabolism, development, locomotion, etc., and these effects are modified by other environmental factors.

Again a monofactorial laboratory approach can give a distorted view of just how the parameter will influence the organism in the field and accordingly such results must be examined cautiously.

Oxygen is usually uniformly distributed through rockpools, the degree of saturation being a function of temperature and salinity. Supplied from the atmosphere and mixed by wind driven turbulence oxygen is usually in adequate supply in rockpools. However, given the presence of even moderate quantities of algae oxygen values fluctuate markedly. Pyefinch (1943) reports a saturation value of 271% at mid-afternoon in a Welsh rockpool. Stephenson, Zoond, and Eyre (1934) working in South Africa report a range between 1.2 and 26.2 mg. O_2/l. (18-278% saturation). In the Baltic the range can be 0.2 to 29.1 mg. O_2/l. (3-361% saturation) (Ganning, 1971a). Lowest values usually are to be found just before sunrise and highest figures in the early afternoon. This emphasizes the point that to be effective monitoring of rockpool environmental factors must be done over short as well as long time intervals.

The greater the density of algae the more variation is possible not only in oxygen but also in pH because of uptake of dissolved carbon dioxide. Values as high as pH 9.3 are given by Pyefinch (1943) and a maximum value of 10.3 in the Baltic (Ganning and Wulf, 1969), both on sunny summer days. Winter diurnal values undergo only minor fluctuations around pH 7 while changes usually reach two units in the summer.

The specific influence of the variations in oxygen and pH values on rockpool organisms has not been studied. Ganning (1971b) did show that the ostracod, Heterocypris incongruens was able to tolerate values as low as 4.4% saturation, but it is normally associated with bottom sediments. The nocturnal migration of some invertebrates including Heterocypris sp. and Gammarus duebeni is not understood. Perhaps low oxygen values serve as a cue or orthokinetic instigator to cause the animals to move to higher oxygen concentrations at the surface away from algal mats. More simply they may avoid daylight and in its absence extend their ranging incidentally coming into contact with higher oxygen values.

Brief mention should be made of the green algae Enteromorpha intestinalis. In most rockpools studied, especially those in the high or supra-littoral zone, it is the dominant algae. For herbivorous and omnivorous members of the fauna Enteromorpha can provide a good food supply throughout the year. In the winter when detrital bottom deposits are commonly low or washed away it is especially necessary, but at other times it provides the fixed carbon and nitrogen source for substrate bacteria, protozoans, and rotifers that in turn are utilized by organisms higher in the food web. Clark (1968) and Vittor (1971) state that primarily because of the presence of this algae, food was never thought to be a limiting factor in pools they studied. Physical factors such as temperature-salinity combinations or tidal inundations were thought to be the main causes of variations in population numbers. *Enteromorpha* also serves as substratum, food, and breeding site for the salt water mite, *Hyadesia fusca* (Ganning, 1970).

There are pools where *E. intestinalis* does not grow and yet still support very dense copepod populations. Detritus of terrestrial or marine origin may provide the necessary nutrients to establish a microfaunal food base. Diatoms could likewise figure in the food web. Further investigations may provide answers.

In describing the fauna of rockpools the distinction can be made between permanent and accidental inhabitants. Most accidental members are species which never inhabit supra-littoral pools but are washed in from the sea on surrounding rocks by tides, storms, or rainwater runoff. Fluctuating environmental conditions soon exceed their tolerances and they die. An example would be the settling of *Balanus* or *Mytilus* nauplii in a pool in the spring but dying as summer temperatures and salinities became unfavorable. By the same token, permanent rockpool residents can be carried to other pools or to the sea where they do not normally form part of the population. Being euryhaline and eurythermal these organisms usually have no trouble with physical conditions. However, the increased stability of lower pools and the ocean allows for greatly increased species diversity and this creates unfavorable biological conditions, i.e., predation, competition. It seems unlikely that great annual and daily fluctuations in physical parameters is necessary for the existence of rockpool organisms in the physiological sense. Rather their tolerance of these fluctuations give permanent residents a secure niche whereas to other organisms these conditions are rigid barriers.

Low species diversity tends to produce fairly simple food webs, but this does not imply that productivity need be low. On the contrary primary production in the summer in Swedish rockpools has been shown to equal that of high productivity tropical environments (Ganning and Wulf, 1969). From this base and given the short life cycle and rapid development to sexual maturity it is quite feasible for T. brevicornis to reach the density of 8900 per liter cited previously.

Because of their position relative to the sea and their generally restricted volumes rockpools are quite unique ecosystems. The sudden and violent fluctuations in abiotic parameters are characteristic of this environment. The great annual and diurnal variation in physical factors allow only opportunistic organisms with high tolerances to survive. This then is a prime focus for investigation of organisms successfully adapted to an extreme environment. The present question is to understand the tolerances that enable rockpool species as characterized by Tigriopus californicus to occupy their niche.

MATERIALS AND METHODS

The experimental animal, Tigriopus californicus, came from two areas and the two groups were utilized in different ways. December, 1975 collections were made from an Enteromorpha lined rockpool at Yaquina Head, Newport, Oregon. This pool had a salinity of 30 o/oo, a water temperature of 12° C., and the air temperature was 10° C. These animals were used in investigations of thermal tolerance at various salinities.

The stock population was held in 35 o/oo seawater, in an 18 ± 1° C. constant temperature room for two weeks. Throughout the experiment they were held on a 12 hour dark:12 hour light cycle and fed pulverized fish food. One third of the stock animals were then placed at each of three acclimation temperatures (10, 18, and 25 ± 1° C.) for another week before being transferred to nesting culture dishes (100 mm. diameter x 50 mm. high) containing approximately 200 ml. of saline solutions (10, 25, 35, 50, and 70 o/oo). Animals were washed at least three times with the appropriate saline solution over a 250 micron mesh before being transferred. The range of salinities was obtained by diluting glasswool-filtered seawater with distilled water and by dilution of a stock brine obtained by slow evaporation of filtered seawater. This method of obtaining hypersaline water was used because it more closely paralleled conditions in the field and might provide a better indication of behavior under natural conditions. An American Optical hand refractometer cross-checked with temperature corrected hydrometer readings was used to determine salinity. Accuracy was ± 1 o/oo. The animals remained at the fifteen temperature-salinity combinations for one week before being tested for thermal tolerance. Small amounts of food were available throughout all acclimations and vessels in all phases of experimentation were covered to minimize evaporation.

Testing for thermal tolerance was accomplished as follows: approximately fifty adult animals were withdrawn from the appropriate culture dish and transferred to a test tube (17 x 150 mm.) in approximately 20 ml. of the acclimation saline solution. The test tube was then placed in a rack in a water bath set at an elevated temperature (27-38 $\pm$ 0.3° C.). The variation in these stress temperatures was unavoidable with the equipment available and tended to produce less than exact results at critical points. The stress period was taken as six hours. It was thought that this would be appropriate since a hypothetical warm day would likely have the rockpools under high solar input for about this period. The lag in heating the contents of a test tube from 10 to 34° C. was five minutes. The oxygen content of two test tubes held at 36° C. for six hours with 70 o/oo water and 41 animals was 4.2 and 4.7 mg. O_2/l. as determined by a Yellow Springs Instrument Company polarographic oxygen probe. Extrapolating from saturation tables this is near saturation for this temperature-salinity combination. Oxygen was not thought to be limiting.

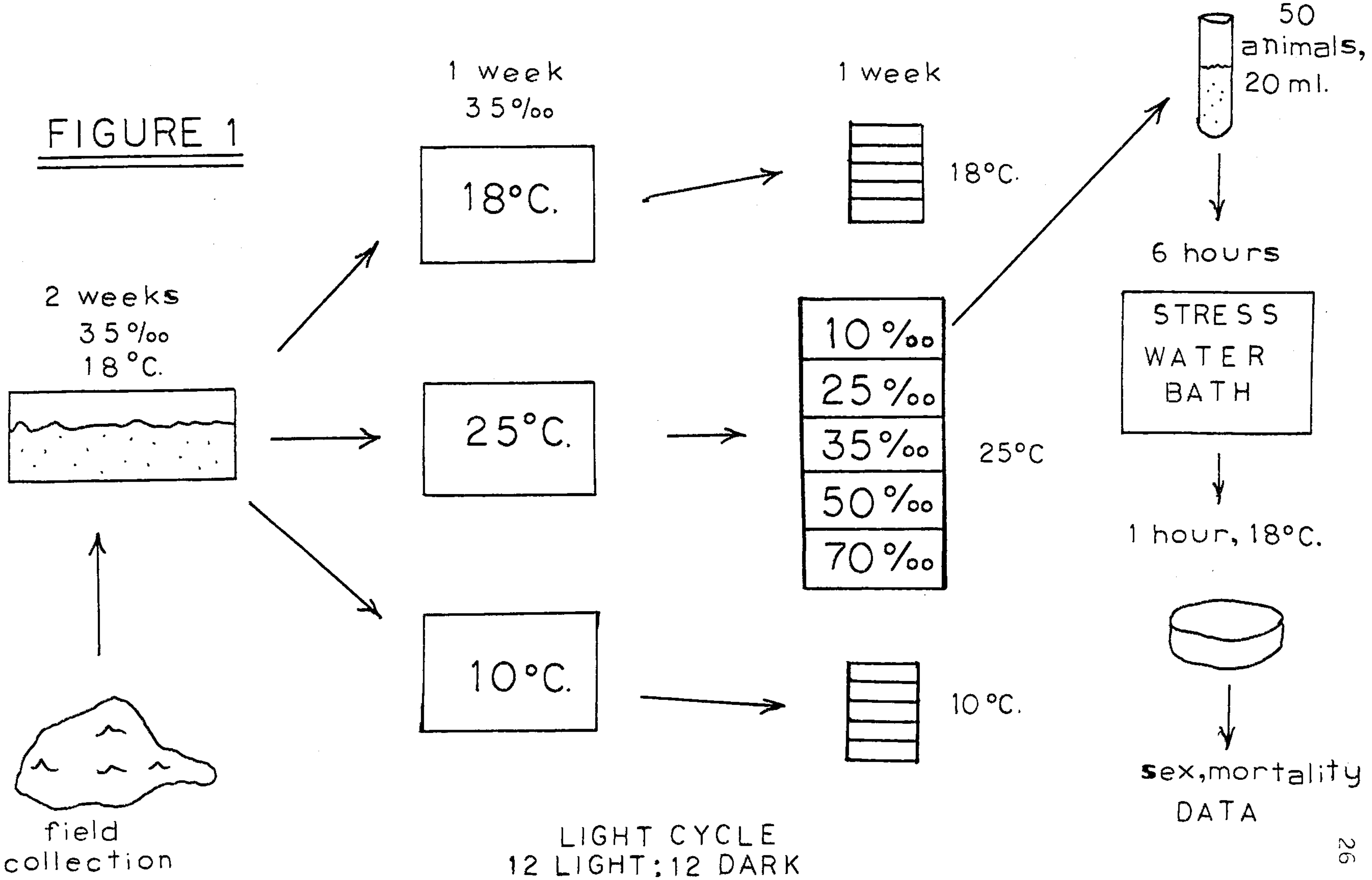
After a six hour stress period the contents of the test tubes were emptied into petri dishes. A one hour refractory period at room temperature was allowed before assessing mortality. Failure to ellicit any movement after prodding with a dissecting needle constituted death. All counts and observations were made on the stage of a dissecting microscope. The experimental procedure is summarized diagramatically in Figure 1.

To evaluate the effects of manipulation control animals were transferred to test tubes in their acclimation saline solutions but held at the appropriate acclimation temperature for six hours. Dead copepods were counted and sexed, and after killing the survivors by dropwise addition of methanol they too were sexed and counted. This applied to the test animals as well.

In examining the joint effects of temperature and salinity acclimation on heat tolerance a total of 311

Figure 1 Experimental scheme for assessing the interaction of tempeature and salinity on heat tolerance.

FIGURE 1


field collection
2 weeks
35‰
18°C.
1 week
35‰
18°C.
25°C.
10°C.
1 week
18°C.
10‰
25‰
35‰
50‰
70‰
25°C
10°C.
50 animals, 20 ml.
6 hours
STRESS WATER BATH
1 hour, 18°C.
sex, mortality DATA
LIGHT CYCLE
12 LIGHT : 12 DARK

replicates were used. Preliminary work indicated that six hour median survival (LD_{50}) varied with acclimation conditions. Initially it was thought that obtaining this figure would be most useful. Consequently replications were concentrated at stress temperatures in the vicinity of the suspected LD_{50} (Table I). Outside this critical area mortality was consistently very low or very high as preliminary investigations demonstrated. Animals and efforts were thought to be best used elsewhere. This arrangement while yielding the information sought did not provide a uniform set of data for all statistical analyses. A foreknowledge of the statistics to be employed would have provided a neater experimental design. To have provided replicates at the missing stress temperatures would have meant new test procedures with new field samples at a later date, i.e., a different experimental population, and this would have been invalid.

_T_. _californicus_ collected in October, 1976 were obtained from a supra-littoral rockpool at Sunset Bay, north of Cape Arago, Charleston, Oregon. At the time of collection the pool had a salinity of 34 o/oo, water temperature was 12.5° C., and the air temperature was 12.0° C. These animals were used to determine salinity tolerance at two temperatures.

The animals were held at room temperature (18-21° C.)

in the Charleston laboratory for one week in 35 o/oo seawater and provided with pulverized fish food. They were then transferred to two sets of petri dishes (90 mm. diameter x 10 mm. deep) containing approximately 30 ml. of saline solutions. These were made by adding glass distilled water to a brine made by mixing Instant Ocean salts and seawater. By salinity determinations described above a series of solutions (distilled water, 5, 10, 20, 30, 50, 70, 80, 90, 100, 110, and 120 o/oo) was prepared.

Approximately 100 adult animals were taken from the stock container and washed as described above before being transferred to a petri dish. One series was placed in an incubator at 26 $\pm$ 1^{o} C. and the other at 8 $\pm$ 1^{o} C., both under an ambient light cycle and without food. The covered containers were checked several times during the first twelve hours and thereafter at 24 hour intervals for a period of 10 days. Lack of food was thought to influence mortality beyond this point. Mortality was recorded based on failure to ellicit any movement after prodding a copepod with a dissecting needle. Dead animals were removed. At the end of the test period survivors were killed with methanol and final counts on males and females tabulated. Preliminary work indicated little variation between replicates accordingly, one sample was used for each temperature-salinity combination.

It was noted that Tigriopus fell into a comatose state within a few minutes of transfer from 35 o/oo to distilled water or 120 o/oo at room temperature (20° C.). If left in these solutions there was no recovery. Groups of 15-20 individuals were removed at intervals from these samples and returned to seawater (35 o/oo) to determine if they could regain normal activity.

RESULTS AND DISCUSSION

Of more than 13,000 adult animals used in the winter salinity-temperature tolerance experiments, 11,574 individuals were sexed, i.e., 3,523 males : 8,051 females. The ratio of males to females was 3:7 which at first seemed odd. However, Shaw (1938) dealt with a California population with a 4:6 ratio, and Vittor's (1971) December field data for Cape Arago show an average ratio of 3:7. Hence, it was felt that the sample was representative of a winter population.

Figures 2-6 show the results of subjecting Tigriopus acclimated to three temperatures (10, 18 and 25° C.) and five salinities (10, 25, 35, 50, and 70 o/oo) to various stress temperatures. The mean mortality for the replicates at a given temperature is plotted on arithmetic paper and the points merely joined. No attempt was made to derive a formula to fit each curve. The inset shows the mean mortality for controls held in test tubes at acclimation temperatures. Table I detailing distribution of replicates shows that emphasis was placed at temperatures producing 25-75% mortality so accurate values of a six hour mean mortality (LD_{50}) could be determined. Statistical breakdown of the mortality data is given in Table II.

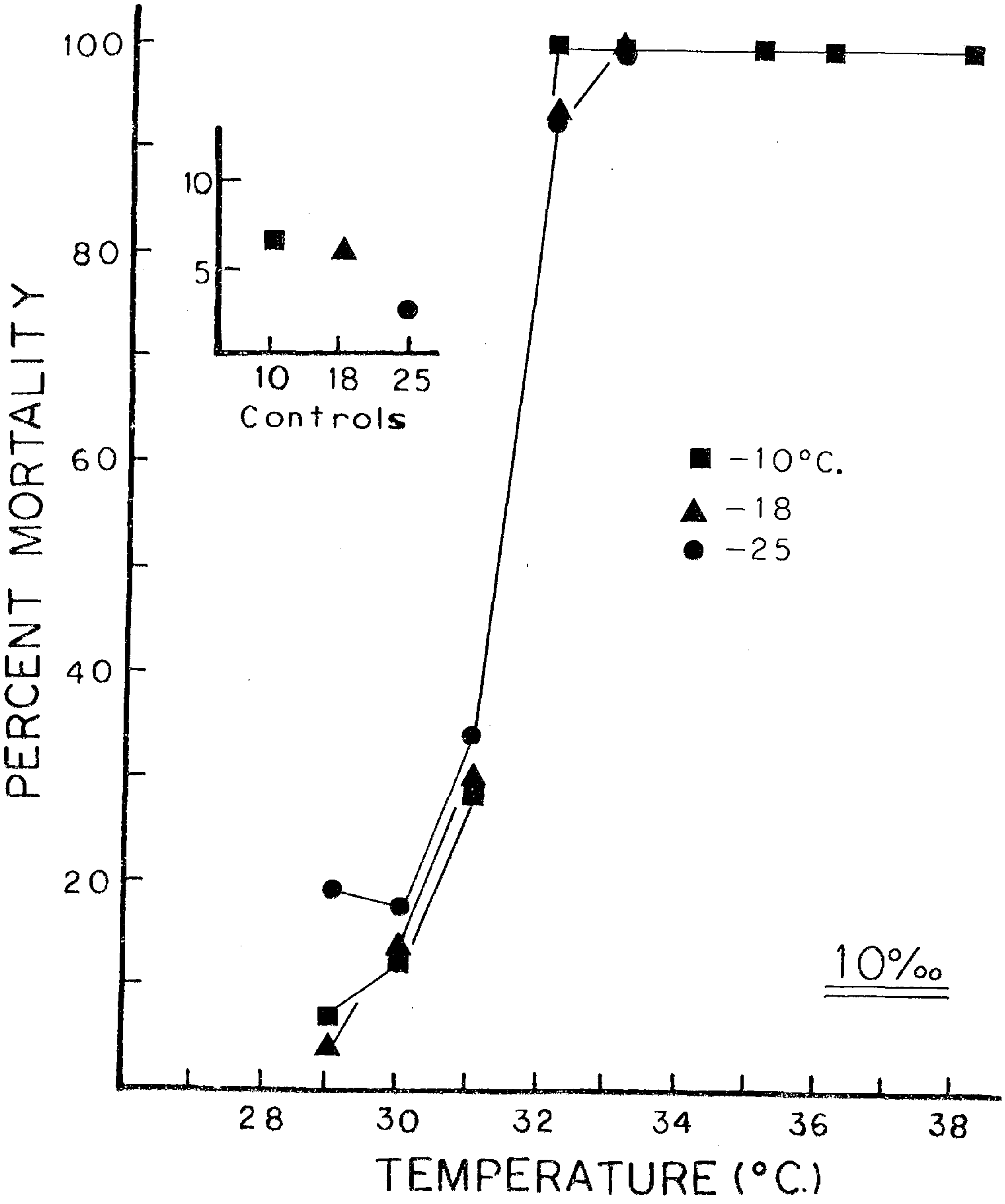


Figure 2 The results of subjecting Tigriopus californicus acclimated to 10 o/oo at each of three acclimation temperatures (10, 18, and 25° C.) to various stress temperatures.

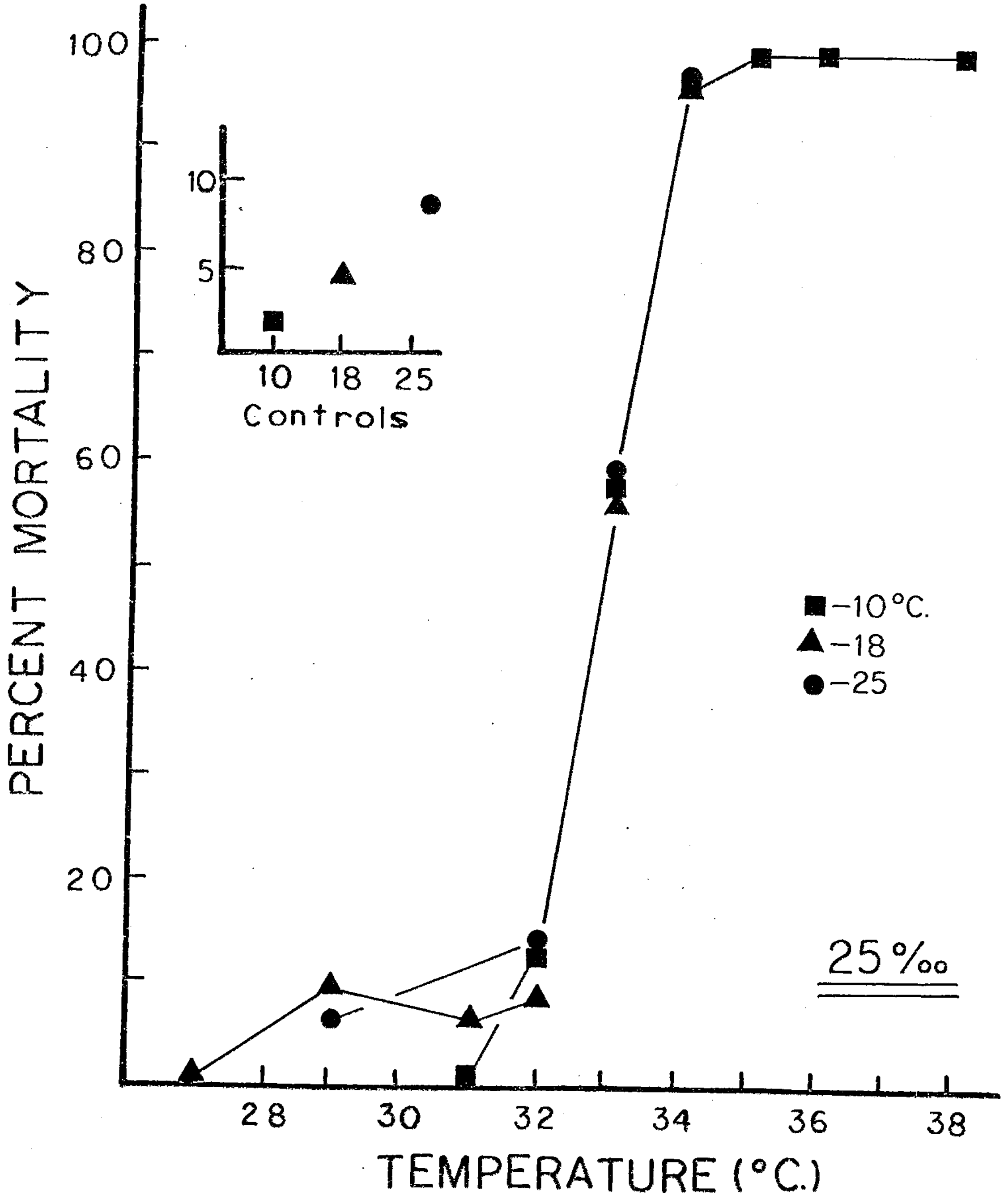


Figure 3 The results of subjecting Tigriopus californicus acclimated to 25 o/oo at each of three acclimation temperatures (10, 18, and 25° C.) to various stress temperatures.

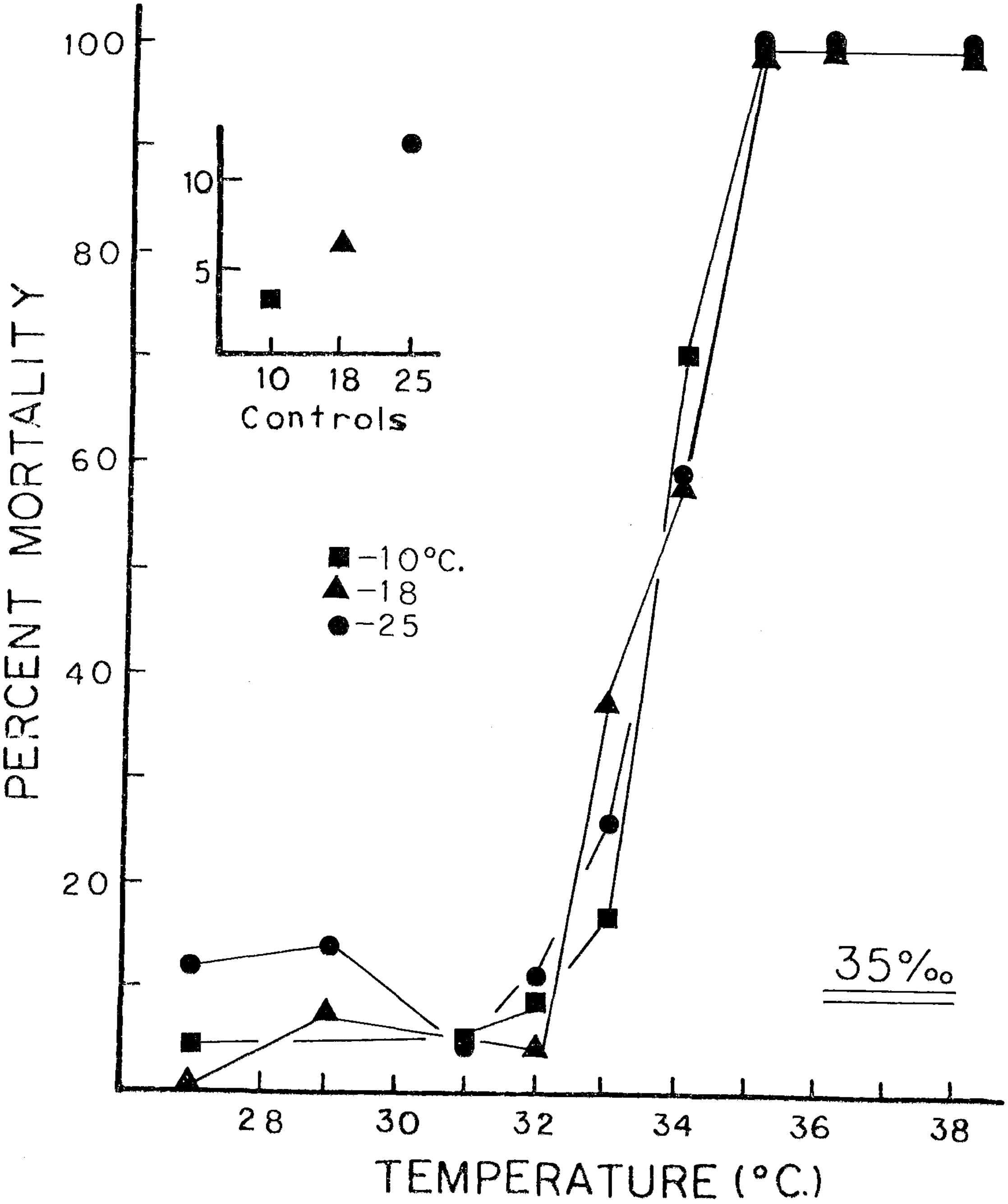


Figure 4 The results of subjecting Tigriopus californicus acclimated to 35 o/oo at each of three acclimation temperatures (10, 18, and 25° C.) to various stress temperatures.

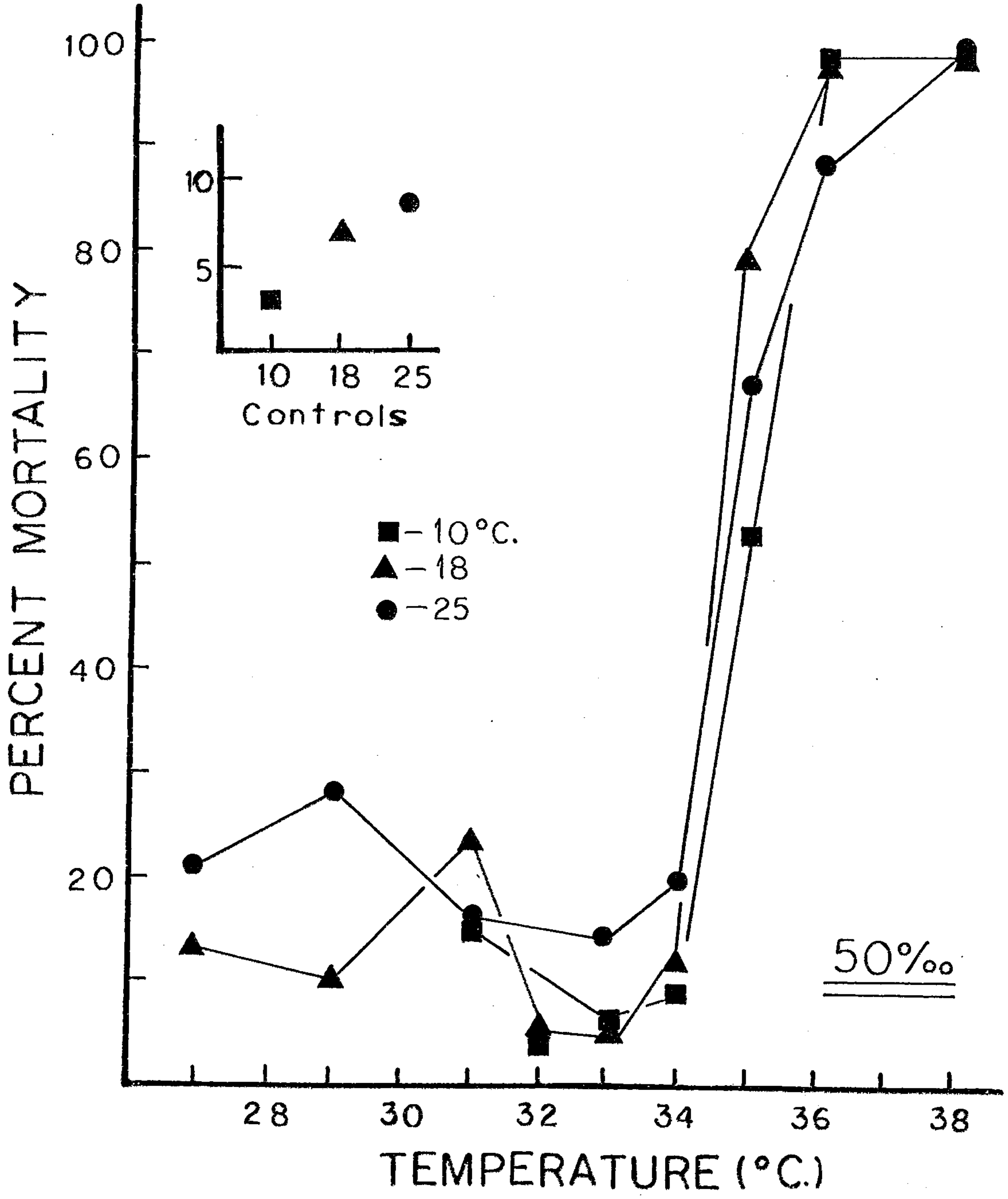


Figure 5 The results of subjecting Tigriopus californicus acclimated to 50 o/oo at each of three acclimation temperatures (10, 18, and 25° C.) to various stress temperatures.

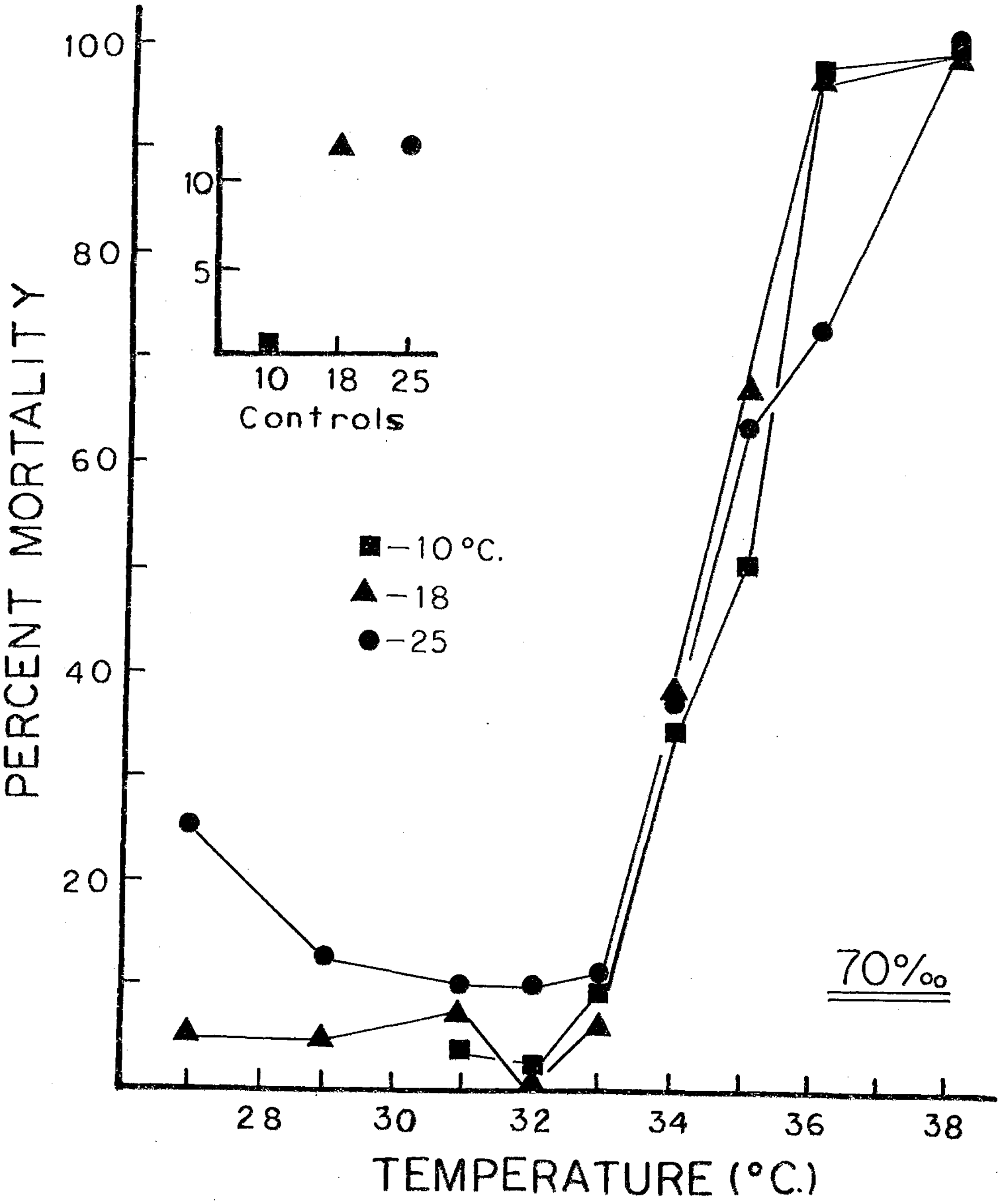


Figure 6 The results of subjecting <u>Tigriopus californicus</u> acclimated to 70 o/oo at each of three acclimation temperatures (10, 18, and 25° C.) to various stress temperatures.

As can be seen from the graphs at all salinities tested there is an abrupt rise in the mortality curve. Such sigmoid curves, implying normal distribution of mortality, with a very narrow critical temperature range have been reported for Hemigrapsus oregonensis and H. nudus where mortality went from zero to 100% over a 2-4° C. range (Todd and Dehnel, 1960). Homarus americanus similarly has a 3° C. critical band (McLeese, 1956). Strongylocentrotus purpuratus exhibited normal behavior at 23.5° C. but at 25 ± 0.5° C. always died (Farmanfarmain and Giese, 1966). In work with Tigriopus brevicornis Ranade (1957) observed the nearly simultaneous death of three-quarters of the animals in his samples once a critical temperature had been reached.

It is believed that the wide, though not usually overlapping, mortality ranges near the LD_{50} are due to the lack of precision of stress temperature water baths. Given the narrow range of critical stress temperature it is understandable that equipment subject to a ± 0.3° C. fluctuation would fail to produce clear-cut results. Nonetheless a sharp upper limit to thermal tolerance existed which seemed unaffected by acclimation temperature at a particular salinity but did vary with acclimation salinity.

This becomes clearer when comparison is made between extrapolated six hour LD_{50} values. These are based on the point at which the mortality curve crossed the line representing median mortality and are given in Table III and graphically in Figure 7. Least squares fit of a polynomial to the points for each of the three acclimation curves generate the equations on the figure. The data show a steady rise in LD_{50} with increasing salinity until 50 o/oo. After this point it appears that the curve falls off. The fitted curves show maxima near 63 o/oo with a decline thereafter. Preliminary work at 90 o/oo give indications of low heat resistance; but due to acclimation problems salinities above 70 o/oo were not used in actual experiments. From the information available it would appear that a maximum six hour LD_{50} would occur near 63 o/oo but the actual curve would fall off more quickly at higher salinities than lower values.

Ranade's (1957) often cited study relating salinity and temperature tolerance demonstrates continuous rise in lethal temperature as a function of salinity. No peak or plateau was observed. In his experiments <u>T. brevicornis</u> was transferred directly from seawater to various saline solutions and subjected to a 2° C. per hour temperature increment. Though indicating similar trends the differences in methods makes comparison invalid. Methodological differences also exist in the work of Matutani (1961),

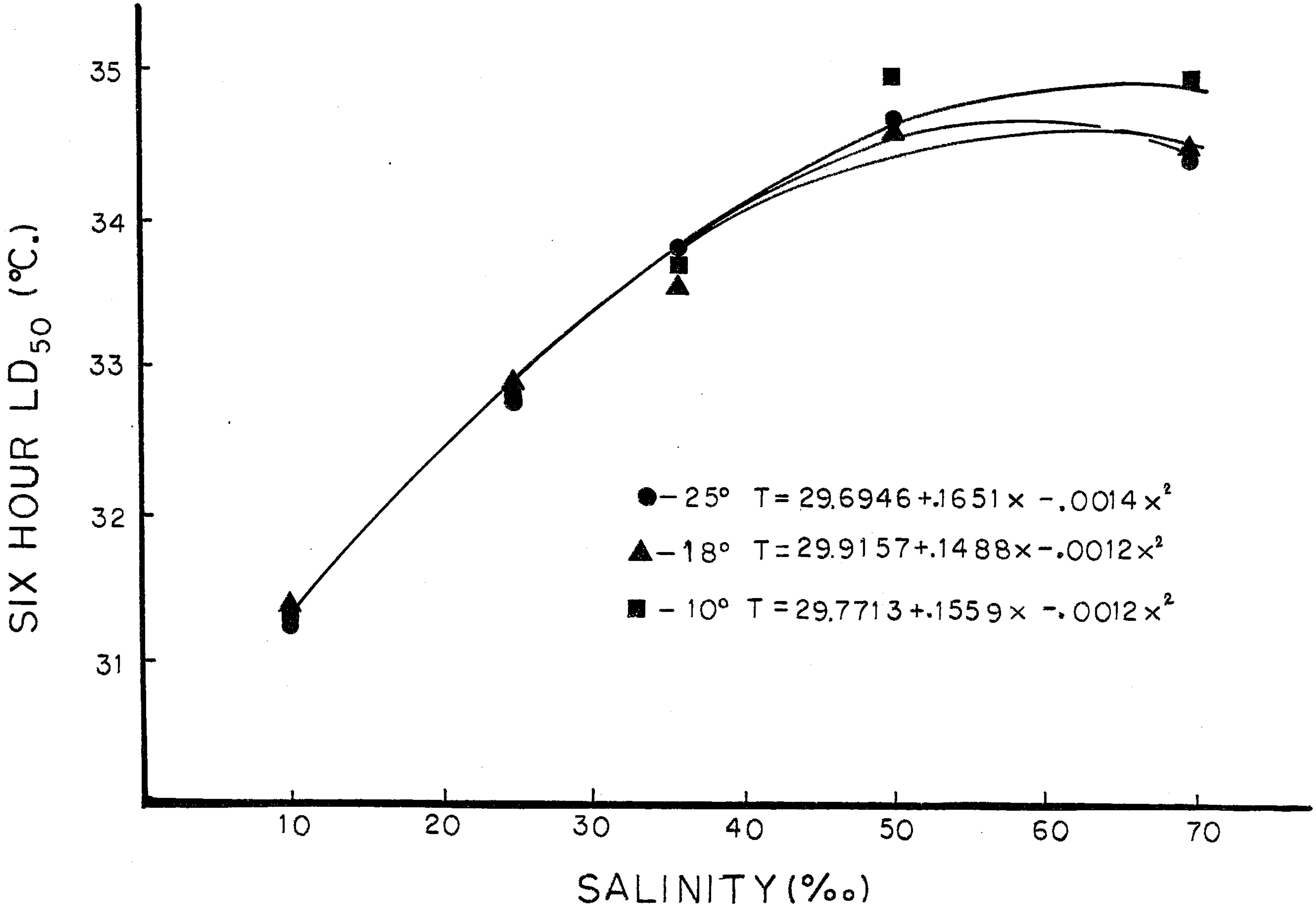


Figure 7 Extrapolated six hour median survival temperatures (LD_{50}) versus salinity for three acclimation temperatures.

but T. japonicus acclimated to a low salinity condition showed decreased heat resistance compared to normal seawater and increased resistance in 150% seawater.

A number of studies support the generalization that acclimation to dilute media tends to shift lethal thermal limits downward and acclimation to more concentrated media tends to shift the limit upward. Acclimation to high salinity increases the resistance of the oligochaete, Enchythaeus albidus, to temperature extremes though the benefit of high salinity was reduced when animals were acclimated to higher temperatures (Kähler, 1970). The tube building polychaete, Clymnella torquata, has low heat resistance in low salinity (Kenny, 1969). In Hemigrapsus nudus and H. oregonensis high temperature, high salinity was the most favorable combination to withstand high test tolerance temperatures while low salinities produced lowest tolerances (Todd and Dehnel, 1960). The heat resistance of the polychaete, Nereis diversicolor, the amphipod, Gammarus duebeni, and the isopod Sphaeroma hookeri is lowered in low salinity adapted animals and raised in high salinity adapted animals (Kinne, 1964). Homarus americanus undergoes systematic increase in upper lethal temperature with increases in acclimation salinity (McLeese, 1956). The calanoid copepod, Eurytemora affinis, demonstrated resistance to temperature extremes at higher salinities (Bradley, 1975). In summary, these animals exhibit enhanced temperature tolerance with increased salinity, at least in the range below stressful salinity.

Taking a different view of salinity-temperature relations Pannikar (1940) working with the shrimps *Palaemonetes varians* and *Leander serratus* and Williams (1960) studying *Penaeus duorarum* and *P. aztecus* found improved survivorship in low salinity-high temperature situations. *Crangon crangon* also showed that with a rise in temperature the salinity optimum was reached by a downward shift in salinity (Broekema, 1941 cited by Todd and Dehnel, 1960). Care should be taken in comparing this low/high information with the high/high-low/low findings above because the criteria for optimum conditions was long life and not thermal tolerance.

As indicated by Table I replicates were not evenly distributed among stress temperatures. This made it impossible to apply an analysis of variance of a complete factorial design to the data as a whole. Had the applicability of this method been realized beforehand the experimental design would have been altered. In the matrix, however, can be found blocks of data suitable for analysis of variance of acclimation temperature, acclimation salinity, and stress temperature on mortality. These figures are enclosed within a dotted boundary and the results of analysis are given in Tables IV-VII. In all cases the effects of stress, salinity, and their joint interaction are highly significant. In only one case is there a significant temperature acclimation effect and in no case are first order effects of acclimation temperature-salinity or acclimation temperature-stress significant. No second order effects of all three factors were observed. Table VI indicates a temperature acclimation effect at stress temperatures 33, 34, and 35° C. and salinities 35, 50, and 70 o/oo. From Figure 7 the most pronounced separation of the curves is in the 34-35° C. range and when this block of data is analyzed (Table VII) no acclimation effect due to temperature is found. Dealing with data in piecemeal fashion is perhaps playing statistical games to make a point and may not be totally valid. However, the cumulative results do seem to reinforce the conclusions drawn earlier from graphs regarding the non-effects of temperature acclimation and the effect of salinity.

The general conclusion that increasing acclimation temperature shifts thermal limits upward and acclimation to low temperature shifts thermal limits downward has been amply documented (Kinne 1963, 1970). Kontogiannis (1973) found this to be the case in _T._ _californicus_ where heat resistance was acquired and lost very rapidly. Matutani

(1961) showed gain in thermal tolerance in both high and low acclimated T. *japonicus* when compared to 20° C. conditioned animals. The present data suggest little or no difference due to thermal acclimation over the range examined. Matutani (1961) showed only slight differences in resistance among 5, 10, and 20° C. acclimated animals; their survival curves sometimes crossing one another. Animals acclimated to 30° C., however, showed pronounced heat resistance. Kontogiannis (1973) showed similar patterns with smaller differences between 10 and 20° C. conditioned animals and larger differences at 30° C. Perhaps then the acclimation range used in these experiments was not sufficiently broad to demonstrate these effects. In this middle temperature range the rapid gain in heat tolerance shown in the first six hours of acclimation by Kontogiannis (1973) could also be producing secondary acclimation to stress temperatures.

Examples do exist in the literature of the non-effects of thermal acclimation. The temperature tolerance of some fish do not seem to be altered by thermal acclimation (Dean, 1972). The grass shrimp, *Palaemonetes* sp. exhibited no temperature acclimation to the temperature regimes tested at 20 o/oo (Thorpe and Hoss, 1975). Acclimation temperature was found to have little effect on the upper limits of thermal tolerance in the copopods *Eurytemora* affinis or Acartia tonsa (Heinle, 1969). Acute experimental conditions were found to influence the respiration response more than acclimation in H. nudus (Hulbert, et al., 1976).

An attempt was made to duplicate the experiment of Kontogiannis (1973) in which he demonstrated acquisition and loss of heat resistance in adult T. californicus acclimated to different temperatures. Winter animals from the Oregon coast suffered very high mortalities using his procedure preventing any conclusion from being drawn. Modification of the temperature regimes in an attempt to increase survivorship did not produce encouraging results and the work was abandoned. The use of different populations subject to different seasonal influences is perhaps the cause of the failure. Further investigation could reveal more concerning acclimation and thermal tolerance in Tigriopus.

The joint effects of temperature and salinity acclimation on thermal resistance for the whole population suggested adult male and female T. californicus were not equally sensitive to temperature stress. Using mean mortalities for stress temperatures where the number of dead males was greater than five, a chi-squared analysis was performed comparing the observed male mortality with the expected mortality derived from the sex ratio of the samples. Table VIII contains the results. The null hypothesis that males and females are equally sensitive to temperature stress is discredited at the 0.1% level. Closer examination, however, reveals that for mean population mortalities less than 50% the results are very highly significant while those cells with population mortalities greater than 50% do not contribute to the significance but rather tend to agree with the null hypothesis. This indicates that the LD_{50} value for males would lie below the population value while that for females would be above this. An example of this greater susceptibility to heat shock is the common observation of amplectic pairs with a dead male being carried around by a viable female. Clark (1968) found similar tolerances in _T. brevicornis_ and _Mesochra lilljeborgi_. Matutani (1961) found female _T. japonicus_ to be hardier. In the copepod, _Euterpina acutifrons_, dimorphic male forms exist, the smaller exhibiting greater high temperature adaptation, but females demonstrated better adaptation to both high and low temperatures than either male form (Moriera and Vernberg, 1968). There was decreased heat tolerance in females of _Gammarus duebeni_ compared to males and no sexual difference was noted in the isopod, _Sphaeroma hookeri_ (Kinne, 1964). Other studies indicate no sweeping generalizations are possible.

Ganning and Wulf (1966) and Ganning (1967, 1971b) have demonstrated preference of various rockpool inhabitants for particular salinities. A field observation gave evidence of this in <u>Tigriopus</u> as well. A through flowing surface layer of fresh water at 15° C. formed a lens over a 42 o/oo pool whose temperature was 28.5° C. <u>T. californicus</u> was observed heavily concentrated at the interface of the two waters apparently balancing out the effects of thermal and osmotic stresses. Examining this preference laboratory animals were placed in a graduated cylinder stratified with three saline solutions (10, 35, and 90 o/oo) made distinct with food colors. Animals oriented quickly to the middle 35 o/oo layer, made occasional brief excursions into 10 o/oo but not the 90 o/oo. Such qualitative observations indicate behavioral mechanisms to avoid unfavorable situations and suggest an area for further investigations.

Neglecting any slight acclimation effects the experimental data has been assembled in Figure 8 to generate a response surface relating mortality salinity, and stress temperature. The three deminsional depiction shows that the sigmoidal shape of the surface does have variations as one moves across isopleths, e.g., the dotted 50% mortality line. The tendency for the surface to fall off at the extreme salinities while having prominence near 60 o/oo would point to sub-optimal and

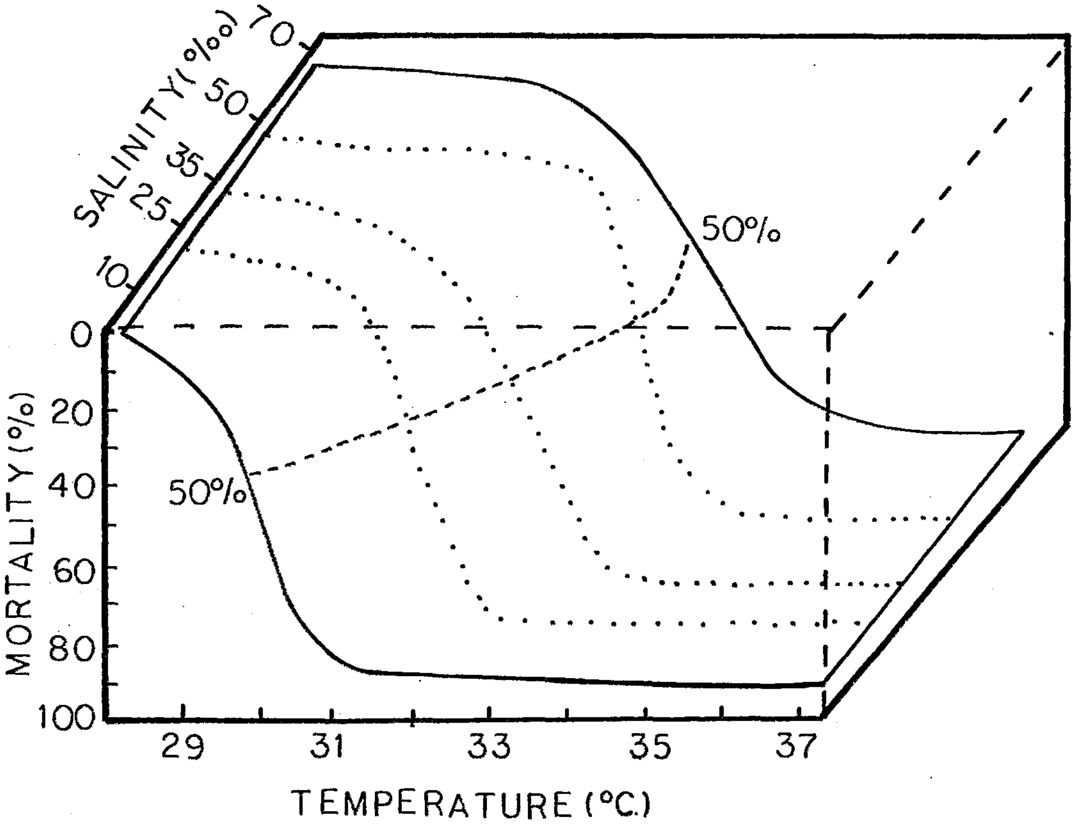


Figure 8 Response surface generated by subjecting <u>Tigriopus</u> <u>californicus</u> to fifteen temperature-salinity combinations and various stress temperatures.

optimal values. The steepness of the slope indicates where interacting factors become especially critical.

Based on the data obtained from salinity and thermal resistance a set of isopleths for mortalities of 10, 50, and 100% are given in Figure 9. Essentially these are the specified mortality contours from Figure 8. Using such a factorial design predictions on the effects of particular combinations of temperature and salinity on the mortality of Tigriopus can be made. Admittedly a limited range is depicted and far-reaching conclusions as drawn by Costlow (1967) and Costlow and Bookhout (1959) with similar effects on crab larvae or by McLeese (1956) working with Homarus americanus cannot be reached. Nonetheless insights into the joint interaction of temperature and salinity can be seen in these depictions which are not obvious with other methods. To more effectively characterize the zone of tolerance for Tigriopus a wider range of temperatures and salinities needs be examined.

One difficulty in the use of these isopleth diagrams is the lack of a time component. In the present case all mortalities relate to an arbitrary six hour stress period. When dealing with success or failure of an organism a meaningful time factor should be included, i.e., relation to tidal conditions in the intertidal, seasonal conditions in an estuary, or reasonable exposure to elevated

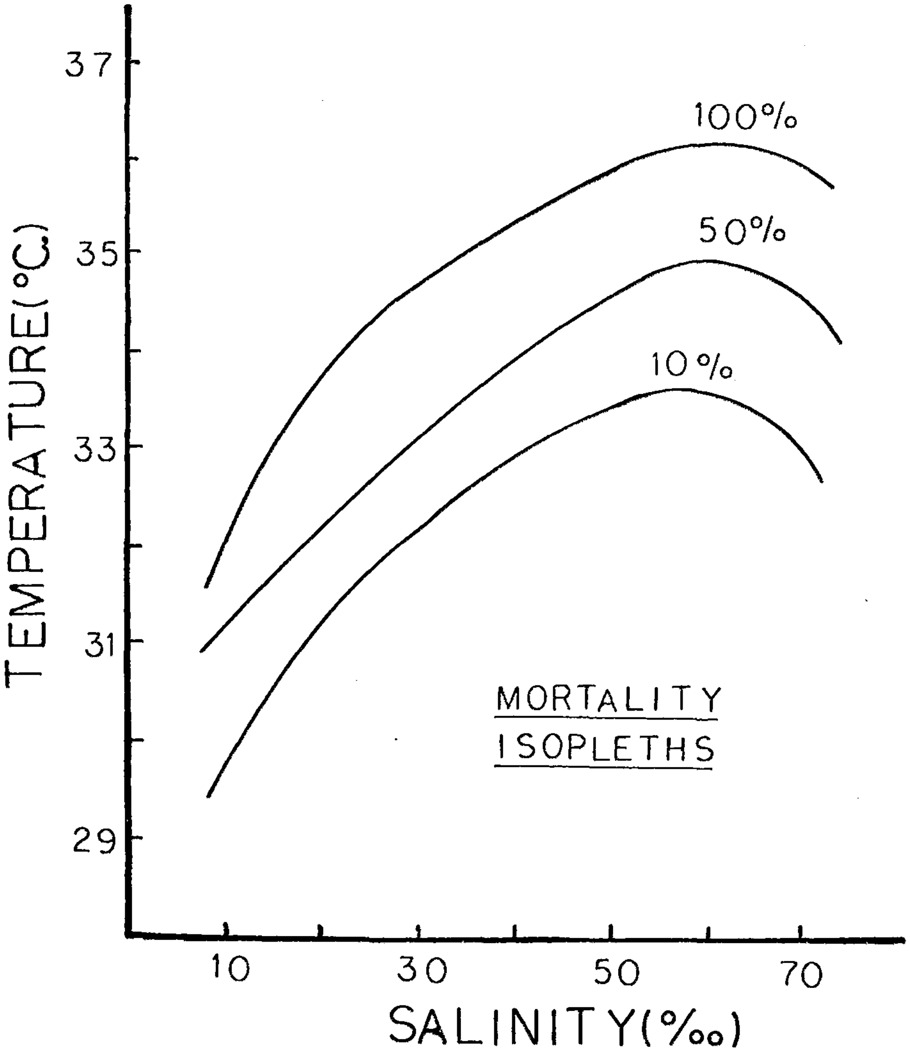


Figure 9 Three mortality isopleths for *Tigriopus californicus* exposed to temperature-salinity interactions.

temperatures. Since the tolerance described can be a function of stage of development, sex, age, season, environmental history, or nutritive state it would be desirable to have a measure of tolerance for all life stages interacting with pertinent environmental factors. Especially in the case of economically important organisms one should ask, which are the most sensitive and which are the most tolerant life stages with regards to certain key factors?

The results of the experiment to determine the upper and lower lethal salinities of T. californicus when stored at two temperatures without food have been expressed as the time taken to reach 50% mortality at each salinity and temperature. This figure was obtained from the plotted cumulative mortality extrapolating the median mortality from the abscissa (Figure 10). Table IX gives the numbers used in plotting Figures 11 and 12.

As shown above females demonstrate greater resistance to salinity in both series. Clark (1968) noted similar results with T. brevicornis. At low temperature the female copepods can effectively survive a range of salinities from 10 to 100 o/oo and males from 30 to 90 o/oo. It should be noted, especially at higher salinity values, both moved very sluggishly and were often upside down with only feeble appendage movements. The former may

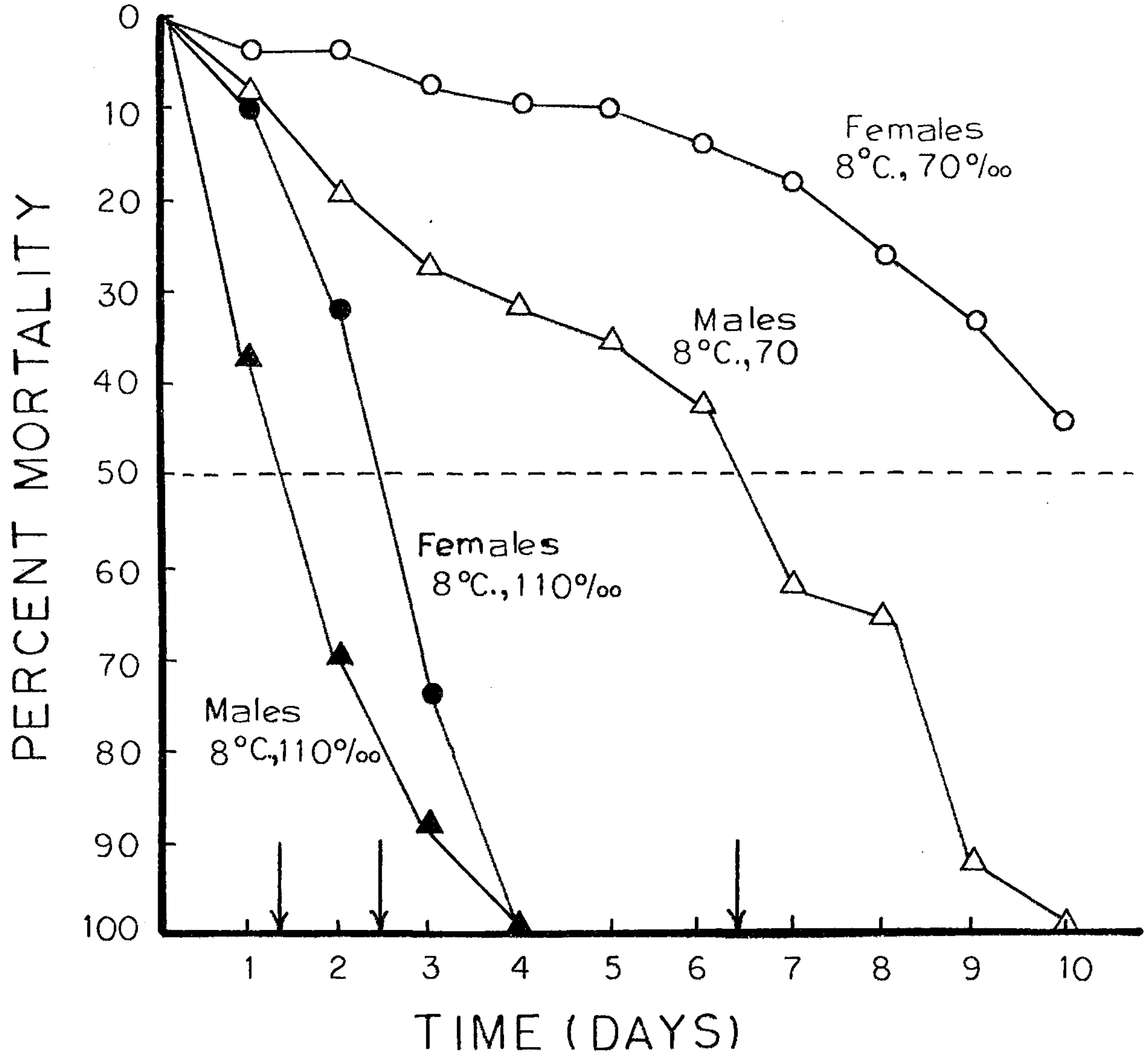


Figure 10 Time-mortality curves for *Tigriopus californicus* held in two salinities (70 and 110 o/oo) at 8° C.

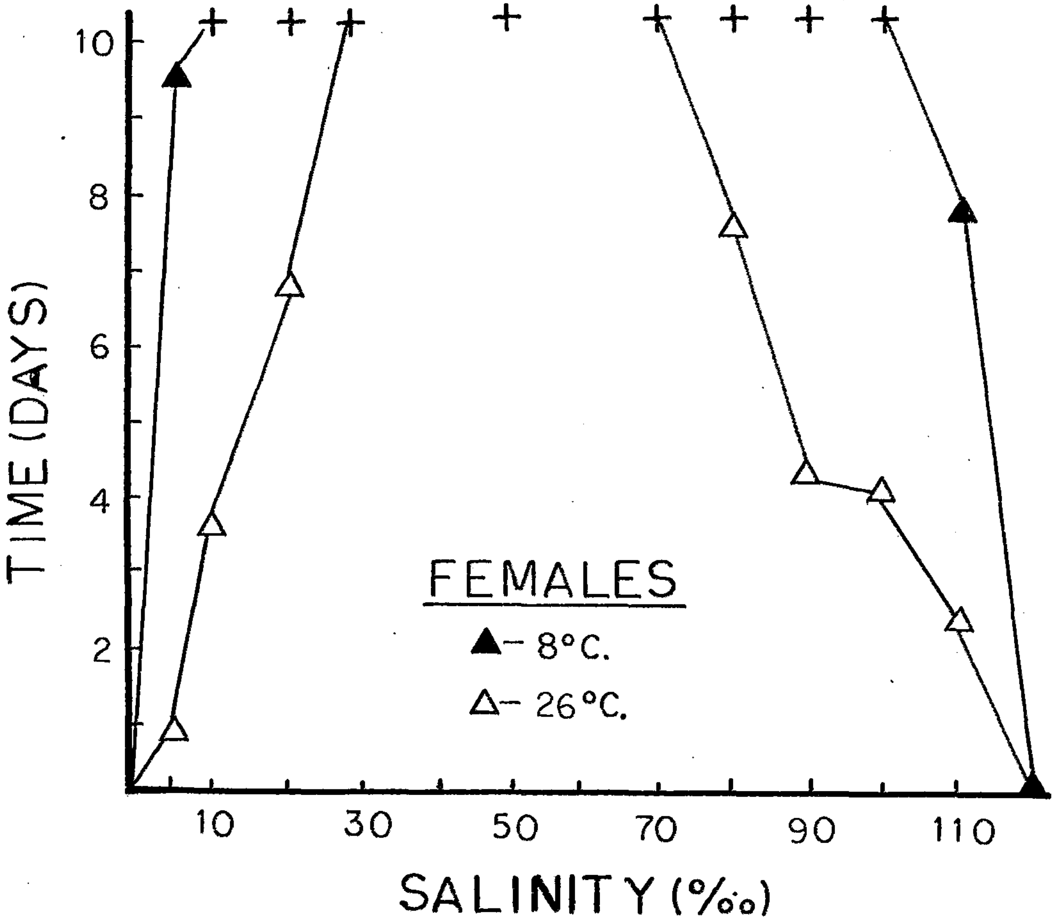


Figure 11 The effect of salinity on median survival time (LD_{50}) for female Tigriopus californicus at two temperatures (8 and 26° C.).

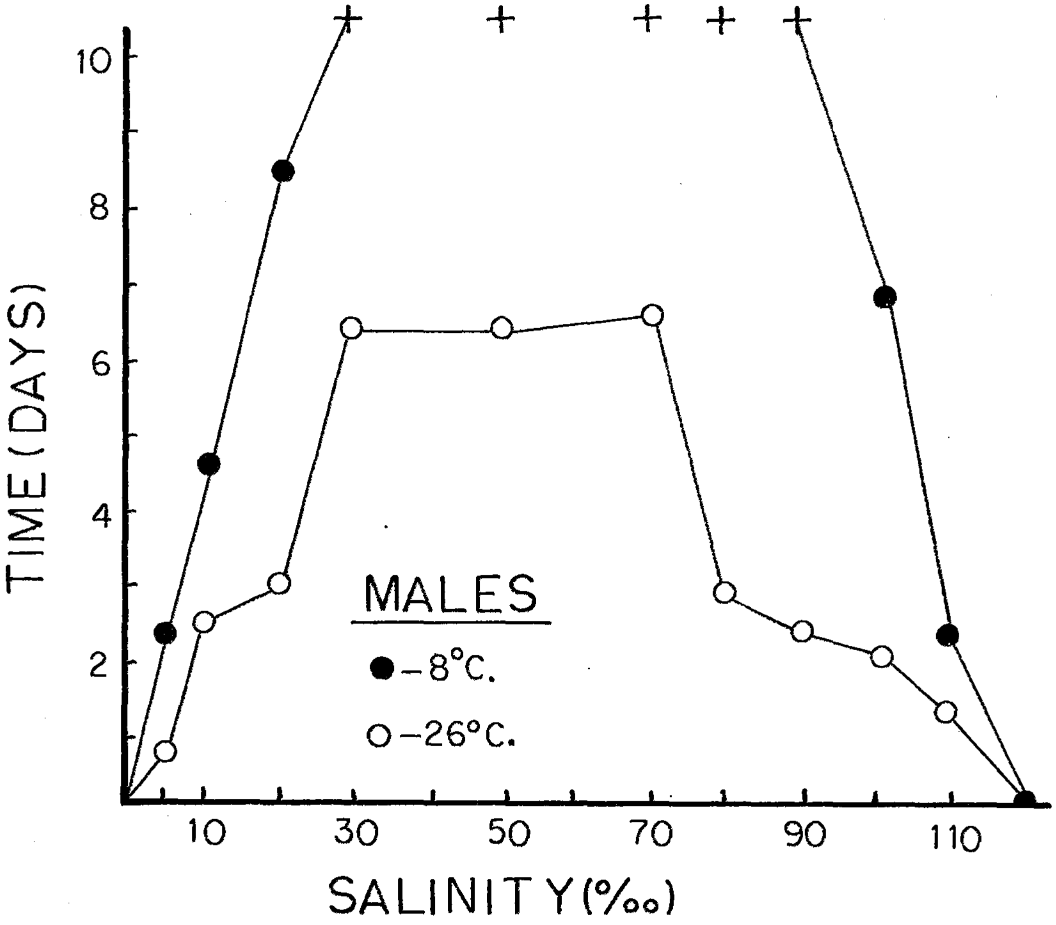


Figure 12 The effect of salinity on median survival time (LD_{50}) for male *Tigriopus californicus* at two temperatures (8 and 26° C.).

have been caused by the increased density of the media and/or osmotic stress. So while viable the behavior of the animals could not be called normal at elevated salinities. No visible swelling or shrinking of Tigriopus was observed as reported by Moriera (1975) with Euterpina acutifrons.

At 26° C. behavior was more normal, but the tolerance range was reduced (30-70 o/oo for females only). As in the first experiment deaths occurred more quickly in low salinities than high salinities. Females were observed to release egg sacs prematurely in hypersaline water. Clark (1968) found that only female T. brevicornis would survive 33.5 o/oo at 25° C. while at 5 and 10° C. the range for both sexes was 9-86 o/oo.

Compared with marine copepods tested at ocean ambient temperatures these ranges are quite impressive. Calanus finmarchicus was found to have a tolerance range of 29.0-55.3 o/oo, Pseudodiaptomus serricaudatus 10.8-43.8 o/oo and Acrocalanus gibber 32.95-35.30 o/oo (Sewell, 1948). Marshall and Orr (1972) found by gradually lowering the salinity Calanus finmarchicus could survive at 12-17 o/oo but behavior was sluggish.

Experiments where salinity has been gradually increased or decreased over a period of days allowing for slow acclimation have extended maximum and minimum salinity limits. A salinity of 11 o/oo was lethal to Acartia lilljeborji acclimated to high salinity but those acclimated to lower salinities could survive dilutions to 6.3 o/oo (Tundisi and Tundisi, 1968, cited by Moriera, 1975). Acartia bifilosa without acclimation exhibited a 50% mortality at 8 o/oo. With slow acclimation over a ten hour period this 50% mortality had been lowered to 2.5 o/oo, thus, narrowing the lower lethal range (Lance, 1963).

It is quite likely that experiments conditioning T. californicus to different salinities would have produced different mortality responses and extended the tolerance range. Laboratory data indicate animals suffer high mortality at 5 o/oo, 8° C., but winter field data (Vittor, 1971) show Tigriopus present in densities of 200 per liter in rockpools at 2 o/oo, 12° C., perhaps the net result of selection. December 1975 animals used in the first experiment were observed to suffer some mortality during acclimation to 10 o/oo, 25° C. relative to other acclimation conditions but not to the extent shown in the salinity tolerance experiment with October 1976 animals. This may indicate seasonal physiological differences as those found in Hemigrapsus oregonensis and H. nudus (Todd and Dehnel, 1960) or the fact that selection has already operated to produce a hardier winter population. Examining a population grown for several generations under stable laboratory conditions as did Matutani (1961) may have eliminated these discrepancies due to natural selection. Disparities between field and laboratory data indicate that total ecological relationships are more complex than laboratory studies of a limited scope will estimate. Non-correspondence can be useful in pinpointing aspects in need of further investigation.

Though unacclimated animals placed in distilled water and 120 o/oo at 18° C. quickly succumbed to the stresses and could be termed "dead" by the experimental critereon it was noted they were capable of resuming normal activity when placed in a 35 o/oo solution. Recovery from this comatose state varied with the solution and the length of immersion. A 50% recovery from distilled water was possible after 30 minutes whereas in 120 o/oo a 50% population mortality was not reached for 3.5 days. In the latter case males exhibited less hardiness than females. Ranade (1957) found T. brevicornis capable of maximum immersion times of 60 hours at 98 o/oo and 30 hours at 135 o/oo before no recovery took place.

Temperature and salinity tolerance can be used as experimental criteria for the demonstration of physiological change. Holding organisms at constant parameter levels for a given time yields data which is easily comparable. More gradual increases or decreases in the factor or cyclic variations are usually closer to natural reality. However, due to differences in the rate of change, results found in the literature are not readily comparable. Tigriopus is certainly subject to violent shifts in physical environment such as sudden changes in salinity due to a heavy rain or tidal inundation, but more gradual changes over a period of hours or days seem more common. As shown above T. californicus can endure exposures to environmental parameters within the lethal zone provided the exposure does not exceed certain finite limits which depend on the level of the parameter. Thus, the animals may be able to survive "indefinitely" at unfavorable salinity-temperature combinations if short time fluctuations into the normal range allow recovery from coma and time for feeding.

An understanding of the potential of organisms to survive fluctuations in both normally encountered environmental factors as well as those induced by man becomes valuable in light of the conflicts between man's use of the ocean for various purposes. For example, thermal addition to an estuary from a power plant could sufficiently reorganize species composition to greatly modify the ecosystem if the thermal tolerance of crucial species was not taken into account. Entrainment in power plant cooling systems subject organisms to short exposures of high temperatures. Knowing the range of tolerance to these brief fluctuations would allow modifications minimizing environmental impact (Ginn, et al., 1976).

Jones (1975) demonstrated the toxicity of cadmium and lead to marine and estuarine animals was enhanced in combination with stressful conditions of salinity. Marine species living at their normal salinities are less susceptible to heavy metal pollution than are estuarine species living near the lower limit of their normal salinity range (Jones, 1973). Fiddler crabs are much more sensitive to mercury when stressed at high temperature and low salinity than when factors were optimal (Vernberg and Vernberg, 1972b).

This information regarding the synergystic action of natural and man-induced factors indicates the need for widely encompassing criteria for environmental quality. Water quality standards should be revised to include the narrowed limits placed on organisms when stressed in a multifactorial manner.

A critical salinity boundary of 5-8 o/oo separates freshwater and marine faunas, a concept which has been documented by Khlebovich (1969). Examination of a number of physiological factors is the basis of this idea, e.g., body fluid concentration of brackish water animals may be decreased to a salinity of about 5 o/oo before serious damage results; below 5 o/oo cellular electrochemical properties and the tissue albumin fraction undergo a marked change (Vernberg and Vernberg, 1972a). Above 5-8 o/oo tolerant poikilosmotic organisms survive, but below this regulation seems to be required. Therefore it is likely that *Tigriopus* can hyperosmoregulate in low salinities.

At high temperature-high salinity conditions in laboratory experiments energy seems to be diverted away from reproduction and presumably to osmoregulation (Vittor, 1971). He inferred successful regulation from the general insensitivity of most fitness related characters of *T. californicus*, i.e., development time, egg size and longevity, to less extreme combinations of temperature and salinity.

The existence of mechanisms of absorption and excretion of salts against considerable gradients is only to be inferred from these considerations. Further bases of euryhalinity could be high tissue tolerance to fluctuations in blood osmoconcentration, genetic plasticity, and behavior. Reduction of contact with the environment by secretion of mucus is a common response of organisms under salinity stress (Kinne, 1964). The mesopsammic copepods studied by Jansson (1968) secreted mucus at low salinities. In these experiments Tigriopus when exposed to extreme salinities was observed to produce some sort of mucus presumably to lower the permeability of the integument.

An organism is a composite of various functional parts acting together in a complex integrated manner. Failure of any one part can cause death. The causes of thermal death are varied. High temperatures tend to cause insufficient oxygen supply and failures in process integration. Enzyme inactivation exceeds reactivation and synthesis. Protoplasmic viscosity increases and changes occur in lipid states. Cell membranes become increasingly permeable (Kinne, 1970). Causes of death due to extreme salinities seem primarily related to osmotic phenomena such as disturbances in the water and mineral concentrations in body fluids and cells and with secondary effects associated with some of the above. It appears the effects of salinity and temperature are similarly related. When salinity is unfavorable then temperature tolerance is decreased and vice versa. Conversely when both salinity and temperature are within the satisfactory range there appears to be no significant interrelationship. Sub-lethal effects on longevity, reproduction, locomotion and development are, however, influenced within narrower limits than tolerance.

The lethal or near lethal temperatures in the tolerance experiments are undoubtedly sufficient in themselves to cause a marked strain on the life processes of Tigriopus, and the additional strain of dealing with a large gradient between body fluids and the external medium at extreme salinities results in animals dying at temperatures lower than when salinity lies at an optimal value.

It may be that modification of the usual intracellular environment by salinity stress could readily cause unstabilizing influences in the structural arrangements of critical proteins. This could make them more susceptable to denaturation. However, elevated salinities somewhere below the lethal or stressful level may alter the intracellular environment to stabilize protein stucture (Brock, 1969). Indirectly by decreasing intracellular water thus decreasing water bound to proteins these proteins may assume greater thermal stability. Animals which tolerate severe freezing produce large pockets of intracellular ice. Markedly reduced metabolic rates with Q_{10}'s as high as 50 may quite possibly be related to the increase in salt concentration of the tissues (Vernberg and Vernberg, 1972a). Increased salinity may offer stabilizing or moderating forces at certain levels.

If salinity is low, protein stability may already be reduced by an increase in molecularly bound water or changes in its distribution due to increased water content. Elevated temperatures could then more quickly have a disruptive effect (Kinne, 1964).

It can be assumed that selection would have acted to produce enzyme systems that would be fairly stable at high temperatures in Tigriopus relative to more stenothermal marine or littoral copepods. The intertidal mollusk, Modiolus desmissus, known to be heat resistant, has aspartic-glutamic transaminase that showed no reduction in activity after twelve minutes at 56° C. (Read, 1963). Working with Hemigrapsus nudus Hulbert, et al. (1976) suggest the enzyme system involved in meeting osmoregulatory stresses possess characteristics which free it from thermal modulation. Thus regardless of the season the animal's response to osmotic stress will be freed from constraints of thermal history. These types of adaptations would be beneficial to organisms with marine affinities coping with near terrestrial temperatures.

Though the physiological limits examined in the experiments may occasionally make their effects known in the environment, it is much more likely that stresses will exert sub-lethal effects. Both Clark (1968) and

Lance (1964) found a direct effect of salinity on fecal pellet production which was taken as a measure of feeding rate. Observations on T. californicus indicate that though activity remains normal in hypo- and hypersaline water fecal pellet production is considerably less than mid-range salinities. Comita and Comita (1966) have shown that 38% of ^{32}P fed to female T. brevicornis is channelled into egg production. Accordingly reduction in feeding rate would result in a decrease in egg production causing a drop in population numbers.

In similar fashion, as noted above, within a wide range of salinities, development time increases with decreases in temperature while longevity increases (Comita and Comita, 1966; Vittor, 1971). Euterpina acutifrons exhibits a longer life cycle at low versus high or normal salinities (Moriera, 1975). Perhaps as reported by Marshall and Orr (1972) cold conditions tend to produce larger individuals than warm conditions and this in the case of females would be translated into increased egg carrying capacity. These facts are useful in examining overwintering Tigriopus populations.

Winter tidal inundations, storms, and rainwater runoff serve to physically remove not only part of the population but the bottom deposits which provide an important food source. Tigriopus is observed to cling tenaciously to the rockpool substrate in crevices and depressions so that even a forceful jet of water from a large collecting syringe could not dislodge the animals. Thus, there is a means for some of the population to stay in the pools. Clark (1968) makes the observation that in winter populations male *T. brevicornis* were absent or scarce and the population was made up of nauplii and females with or without egg sacs. The low percentage of males in Oregon rockpools in winter seem to follow a similar pattern most likely because of selection due to low salinities.

Low winter temperatures and salinities would be a favorable resistance combination and one which would also enhance longevity. Reduced food resources may inactivate general metabolism and thus conserve energy. When more favorable conditions return in the spring the increase in available energy could be directed toward egg production. Since the transfer of a spermatophore at the first copulation provides for many broods the continual presence of males is not mandatory to insure population growth as conditions warrant. Interestingly Clark (1968) suggests that the first eggs of each generation develop into males. In citing Igarashi (1963) she states that in *T. japonicus* the primary sex ratio is always male dominated, though Vittor (1971) noticed no effect on sex ratio. Regardless, this may allow for a comeback of males eliminated over winter to fertilize new females and allow steady population increases under favorable temperature and salinity conditions found in spring and summer. Resistance to high salinity-high temperature combinations will enable the copepods to cope with summer and fall conditions to a great measure, but evaporation, tidal inundation, or severe dilution accompanied by high temperatures generally begin to reduce numbers until once again a winter minimum is reached. Adverse salinities and tidal action can be critical at any time of year.

Food and other factors may be important in enabling *Tigriopus* to survive adverse conditions for longer periods. Further, acclimation to one factor, e.g., salinity, may proceed at different efficiencies under different levels of other similtaneous acclimations, e.g., temperature and oxygen, and this may help or hinder the capacity of *Tigriopus* for maximal adaptation to existing conditions. Especially at the limits of tolerance the interactions between factors would have the greatest effects. In examining a eurythermal-euryhaline species like *T. californicus* one should consider both the duration and quantity of a factor in assessing its role. When numerous factors are altered simultaneously the organism must possess highly integrative mechanisms to cope successfully and completely with the environmental situation. Species from highly variable environments evolve physiological adaptibility to allow exploitation of available resources, e.g., normal reproduction can take place in spite of sizeable variation in temperature and salinity though it should be assumed that since not all combinations produce a single optimal result, energy is being appropriately allocated to balance needs such as osmoregulation.

The opportunistic life history strategy of Tigriopus with its short generation time, high fecundity, and decreased longevity is well adapted to the high intertidal and supra-littoral rockpool environment. This allows for establishment and rapid growth in pools that may be haphazardly seeded. High tolerance has been selected for, rather than behavior that might result in escape from deteriorating conditions.

Tolerance alone is not sufficient to explain why Tigriopus which can tolerate typical marine salinities and temperatures remains unsuccessful in that environment. Certainly there are adequate opportunities for contact with the sea and the animal possesses great abiotic potential. Most likely then biological limitation is operating at one or several levels. Marine forms are likely to be highly efficient and specific in their niches.

As such a generalist like T. californicus is likely to be outcompeted or fall victim to these more refined ecological specialists. It can, however, attain dominance in an environment that puts severe limitations on specialized forms. As such Tigriopus embodies some of the traits that lead to emmigration from the sea via the littoral zone to the land. Being capable of withstanding fluctuating temperatures and developing tolerance to high salinities would seem to be factors facilitating transition. The hard exoskeleton, strong jointed appendages, and internal fertilization seem positive pre-adaptions to a terrestrial existence. But tolerating the environment rather than controlling it has prevented crustaceans from assuming the dominance of insects, i.e., cuticle to protect against surface evaporation, trachae versus gills, uric acid versus ammonia (Kinne, 1964). Nonetheless, Tigriopus occupying the niche it does indicates trends that may have been in operation in an earlier time and indeed are probably occurring today, i.e., the mud skipper, Periophthalmus.

All living organisms are genetically adapted to their ecological niche in one way or another. Their very presence is evidence for a suitable genetic background. From this background a tolerance zone emerges which can vary with physiological condition, stage of development, sex, acclimation, etc. The range of salinities and thermal limits investigated here show that Tigriopus californicus possesses eurythermal and euryhaline characteristics that cover a broad range of field conditions that effectively exclude other species from successfully occupying its rockpool niche.

APPENDIX

Table I Distribution of replicates used in assessing mortality at various acclimation and stress conditions.

Acclimation Temperature (°C.)	Acclimation Salinity (o/oo)	Controls	STRESS TEMPERATURE (°C.)										Number Replicates
			27	29	30	31	32	33	34	35	36	38	
	10	4	0	2	3	4	3	3	0	1	1	1	
	25	3	0	0	0	2	3	5	5	1	1	1	
10	35	3	1	0	0	1	2	5	3	5	1	1	
	50	3	0	0	0	1	2	4	3	3	2	1	
	70	3	0	0	0	1	1	2	3	6	3	1	
													113
	10	3	0	3	3	3	3	2	0	0	0	0	
	25	3	1	1	0	3	3	5	4	0	0	0	
18	35	2	1	1	0	1	1	4	3	4	1	1	
	50	3	1	1	0	2	2	3	3	3	2	1	
	70	3	1	1	0	2	1	3	3	3	3	1	
													105
	10	1	0	2	2	3	4	2	0	0	0	0	
	25	3	0	1	0	0	4	3	3	0	0	0	
25	35	4	1	1	0	1	1	4	3	4	1	1	
	50	3	1	1	0	1	1	4	3	4	4	1	
	70	3	1	1	0	1	1	3	3	4	3	1	
													93
													311

Table II Statistical analysis of mortality data derived from subjecting *Tigriopus californicus* to fifteen temperature-salinity combinations and various stress temperatures.

Acclimation Temperature (°C.)	Acclimation Salinity (o/oo)	Stress Temperature (°C.)	Mean Mortality (%)	Range (%) (minimum-maximum)	S.D.±	S.E.±
10	10	10	6.72	3.23 - 7.53	3.09	1.55
		29	7.69	0.00 - 15.38	10.87	7.72
		30	12.78	0.00 - 33.33	17.98	10.39
		31	27.67	14.00 - 37.93	9.86	5.91
		32	100.00	100.00 - 100.00	0.00	0.00
		33	100.00	100.00 - 100.00	0.00	0.00
		35	100.00	100.00 - 100.00	0.00	0.00
		36	100.00	100.00 - 100.00	0.00	0.00
		38	100.00	100.00 - 100.00	0.00	0.00
	25	10	2.11	0.00 - 4.00	2.01	1.16
		31	1.22	0.00 - 2.44	1.72	1.22
		32	13.18	0.00 - 37.50	21.09	12.19
		33	57.87	39.02 - 67.57	11.35	5.08
		34	96.93	90.32 - 100.00	3.98	1.78
		35	100.00	100.00 - 100.00	0.00	0.00
		36	100.00	100.00 - 100.00	0.00	0.00
		38	100.00	100.00 - 100.00	0.00	0.00
	35	10	3.33	0.00 - 10.00	5.77	3.34
		27	4.17	4.17 - 4.17	0.00	0.00
		31	3.57	3.57 - 3.57	0.00	0.00
		32	8.50	6.67 - 10.34	2.60	1.84
		33	16.83	0.00 - 25.93	10.38	4.64
		34	70.13	58.62 - 82.54	11.98	6.92
		35	100.00	100.00 - 100.00	0.00	0.00
		36	100.00	100.00 - 100.00	0.00	0.00
		38	100.00	100.00 - 100.00	0.00	0.00
	50	10	3.08	2.86 - 3.45	2.44	1.85
		31	15.38	15.38 - 15.38	0.00	0.00

Table II continued.

Acclimation Temperature (°C.)	Acclimation Salinity (o/oo)	Stress Temperature (°C.)	Mean Mortality (%)	Range (%) (minimum-maximum)	S.D.±	S.E.±
10	50	32	2.83	0.00 - 5.66	4.00	2.84
		33	7.13	3.45 - 14.86	5.23	2.61
		34	10.53	7.89 - 13.27	2.69	1.55
		35	53.17	48.78 - 56.72	4.03	2.33
		36	100.00	100.00 - 100.00	0.00	0.00
		38	100.00	100.00 - 100.00	0.00	0.00
	70	10	0.00	0.00 - 0.00	0.00	0.00
		31	4.17	4.17 - 4.17	0.00	0.00
		32	2.32	2.32 - 2.32	0.00	0.00
		33	9.62	0.00 - 19.23	12.60	9.64
		34	34.71	21.67 - 47.47	9.70	7.46
		35	51.39	31.37 - 82.46	18.00	5.47
		36	98.81	98.08 - 100.00	0.79	0.60
		38	100.00	100.00 - 100.00	0.00	0.00
18	10	18	5.67	2.78 - 8.82	3.03	1.75
		29	4.86	0.00 - 8.93	4.52	2.61
		30	13.07	7.69 - 18.18	5.25	3.03
		31	29.26	21.88 - 36.36	7.25	4.19
		32	92.20	90.48 - 93.10	1.49	8.64
		33	100.00	100.00 - 100.00	0.00	0.00
	25	18	4.92	0.00 - 10.00	5.00	2.89
		27	0.00	0.00 - 0.00	0.00	0.00
		29	10.00	10.00 - 10.00	0.00	0.00
		31	6.90	0.00 - 20.69	11.95	6.90
		32	9.19	3.70 - 18.87	9.41	4.86
		33	56.32	43.47 - 62.50	7.67	3.43
		34	96.98	90.63 - 100.00	4.42	2.21
	35	18	6.25	0.00 - 12.50	8.84	6.27
		27	0.00	0.00 - 0.00	0.00	0.00

Table II continued.

Acclimation Temperature (°C.)	Acclimation Salinity (o/oo)	Stress Temperature (°C.)	Mean Mortality (%)	Range (%) (minimum-maximum)	S.D.±	S.E.±
18	35	29	7.14	7.14 - 7.14	0.00	0.00
		31	0.00	0.00 - 0.00	0.00	0.00
		32	4.17	4.17 - 4.17	0.00	0.00
		33	37.08	19.23 - 65.00	21.26	10.63
		34	57.83	48.48 - 62.50	8.09	4.68
		35	100.00	100.00 - 100.00	0.00	0.00
		36	100.00	100.00 - 100.00	0.00	0.00
		38	100.00	100.00 - 100.00	0.00	0.00
	50	18	6.88	4.55 - 10.20	2.96	1.71
		29	1.11	11.11 - 11.11	0.00	0.00
		31	23.66	18.75 - 28.57	6.94	4.93
		32	5.00	0.00 - 10.00	7.07	5.01
		33	5.54	2.63 - 7.89	2.68	1.55
		34	12.38	1.35 - 24.24	11.47	6.63
		35	79.99	76.00 - 87.50	6.51	3.76
		36	100.00	100.00 - 100.00	0.00	0.00
		38	100.00	100.00 - 100.00	0.00	0.00
	70	18	11.90	8.60 - 16.67	4.22	2.44
		27	5.55	5.55 - 5.55	0.00	0.00
		29	4.76	4.76 - 4.76	0.00	0.00
		31	7.39	5.26 - 9.50	3.01	2.14
		32	0.00	0.00 - 0.00	0.00	0.00
		33	5.78	3.13 - 7.69	2.37	1.37
		34	38.53	35.90 - 42.55	3.54	2.05
		35	65.98	62.00 - 69.77	3.89	2.25
		36	97.00	94.59 - 100.00	2.75	1.59
		38	100.00	100.00 - 100.00	0.00	0.00
25	10	25	2.56	2.56 - 2.56	0.00	0.00
		29	19.84	17.46 - 22.22	3.37	2.39

Table II continued.

Acclimation Temperature (°C.)	Acclimation Salinity (o/oo)	Stress Temperature (°C.)	Mean Mortality (%)	Range (%) (minimum-maximum)	S.D.±	S.E.±
25	10	30	17.40	14.29 - 20.51	3.11	3.12
		31	32.61	28.57 - 39.20	4.28	3.25
		32	92.12	79.31 - 100.00	9.02	4.51
		33	100.00	100.00 - 100.00	0.00	0.00
	25	25	9.17	6.25 - 14.28	4.44	2.57
		29	7.14	7.14 - 7.14	0.00	0.00
		32	12.76	5.13 - 19.44	5.87	2.94
		33	60.23	56.25 - 62.79	3.49	2.00
		34	97.20	94.74 - 100.00	2.65	1.53
	35	25	12.01	5.71 - 18.18	5.25	2.62
		27	11.11	11.11 - 11.11	0.00	0.00
		29	13.33	13.33 - 13.33	0.00	0.00
		31	5.26	5.26 - 5.26	0.00	0.00
		32	11.11	11.11 - 11.11	0.00	0.00
		33	25.76	5.13 - 39.58	15.00	7.49
		34	59.38	42.86 - 85.29	22.72	13.13
		35	98.75	96.77 - 100.00	1.56	0.78
		36	100.00	100.00 - 100.00	0.00	0.00
		38	100.00	100.00 - 100.00	0.00	0.00
	50	25	8.74	4.54 - 14.71	5.31	2.30
		27	21.43	21.43 - 21.43	0.00	0.00
		29	28.57	28.57 - 28.57	0.00	0.00
		31	16.67	16.67 - 16.67	0.00	0.00
		32	6.90	6.90 - 6.90	0.00	0.00
		33	14.14	3.64 - 27.27	10.38	5.19
		34	20.11	18.52 - 22.45	2.07	1.20
		35	66.56	51.85 - 80.65	11.91	5.96
		36	92.67	89.19 - 100.00	3.66	2.51
		38	100.00	100.00 - 100.00	0.00	0.00
	70	25	12.82	3.51 - 30.18	15.05	6.69

Table II continued.

Acclimation Temperature (°C.)	Acclimation Salinity (o/oo)	Stress Temperature (°C.)	Mean Mortality (%)	Range (%) (minimum-maximum)	S.D.±	S.E.±
25	70	27	25.00	25.00 - 25.00	0.00	0.00
		29	11.76	11.76 - 11.76	0.00	0.00
		31	10.00	10.00 - 10.00	0.00	0.00
		32	10.00	10.00 - 10.00	0.00	0.00
		33	10.54	7.28 - 16.67	5.31	3.07
		34	39.86	21.74 - 52.38	16.07	6.98
		35	64.15	50.00 - 76.71	10.96	5.48
		36	72.41	65.79 - 81.63	8.24	4.76
		38	100.00	100.00 - 100.00	0.00	0.00

Table III Extrapolated six hour median survival temperatures (LD_{50}) for fifteen temperature-salinity combination.

Acclimation Salinity (o/oo)	Acclimation Temperature (°C.) 10	18	25
10	31.30° C.	31.33° C.	31.29° C.
25	32.83	32.87	32.79
35	33.64	33.50	33.76
50	34.92	34.57	34.63
70	34.89	34.43	34.38

Table IV Components of three-way analysis of variance of acclimation temperature (10, 18, and 25° C.), acclimation salinity (10 and 25 o/oo) and stress temperature (32 and 33° C.) on mortality.

Source of Variance	df	SS	MS	F
Replication	1	0.000418	0.000418	NS
Main Effects:				
Acclimation Temperature	2	0.003434	0.001717	NS
Acclimation Salinity	1	2.436972	2.436972	650.98*
Stress Temperature	1	0.465560	0.465560	124.36*
First Order Effects:				
Acclimation Temperature-Acclimation Salinity	2	0.002539	0.001270	NS
Acclimation Temperature-Stress Temperature	2	0.006992	0.003496	NS
Acclimation Salinity-Stress Temperature	1	0.325671	0.325671	87.00*
Second Order Effects:				
Acclimation Temperature-Acclimation Salinity-Stress Temperature	2	0.027906	0.013953	NS
Error	11	0.041179	0.003744	-
Total	23	3.310672	-	-

*$p < 0.01$

Table V Components of three-way analysis of variance of acclimation temperature (18 and 25° C.), acclimation salinity (25, 35, and 50 o/oo) and stress temperature (33 and 34° C.) on mortality.

Source of Variance	df	SS	MS	F
Replication	2	0.000220	0.000110	NS
Main Effects:				
Acclimation Temperature	1	0.013306	0.013306	NS
Acclimation Salinity	2	2.852646	0.950882	84.21*
Stress Temperature	1	0.899338	0.899338	79.64*
First Order Effects:				
Acclimation Temperature-Acclimation Salinity	3	0.020646	0.006882	NS
Acclimation Temperature-Stress Temperature	1	0.001588	0.001588	NS
Acclimation Salinity-Stress Temperature	3	0.222288	0.074095	6.56*
Second Order Effects:				
Acclimation Temperature-Acclimation Salinity-Stress Temperature	3	0.007728	0.002576	NS
Error	30	0.338770	0.011292	-
Total	47	4.356530	-	-

*$p < 0.01$

Table VI Components of three-way analysis of variance of acclimation temperature (10, 18, and 25° C.), acclimation salinity (35, 50, and 70 o/oo) and stress temperature (33, 34, and 35° C.) on mortality.

Source of Variance	df	SS	MS	F
Replication	1	0.000431	0.000431	NS
Main Effects:				
Acclimation Temperature	2	0.831360	0.415680	3.97**
Acclimation Salinity	2	0.762042	0.381021	36.43*
Stress Temperature	2	3.741950	1.870975	175.88*
First Order Effects:				
Acclimation Temperature-Acclimation Salinity	4	0.028100	0.007025	NS
Acclimation Temperature-Stress Temperature	4	0.061208	0.015302	NS
Acclimation Salinity-Stress Temperature	4	0.398087	0.099522	9.50*
Second Order Effects:				
Acclimation Temperature-Acclimation Salinity-Stress Temperature	8	0.082789	0.010349	NS
Error	26	0.271937	0.010459	-
Total	53	5.429680	-	-

*$p < 0.01$

**$p < 0.05$

Table VII Components of three-way analysis of variance of acclimation temperature (10, 18, and 25° C.), acclimation salinity (35, 50, and 70 o/oo) and stress temperature (34 and 35° C.) on mortality.

Source of Variance	df	SS	MS	F
Replication	2	0.010175	0.005088	NS
Main Effects:				
Acclimation Temperature	2	0.176683	0.008834	NS
Acclimation Salinity	2	1.627468	0.813734	66.45*
Stress Temperature	1	1.919228	1.919228	156.72*
First Order Effects:				
Acclimation Temperature-Acclimation Salinity	4	0.072974	0.018244	NS
Acclimation Temperature-Stress Temperature	2	0.044914	0.022457	NS
Acclimation Salinity-Stress Temperature	2	0.173756	0.086878	7.09*
Second Order Effects:				
Acclimation Temperature-Acclimation Salinity-Stress Temperature	4	0.027395	0.006849	NS
Error	34	0.416373	0.012246	-
Total	53	4.309952	-	-

*$p < 0.01$

Table VIII Chi-squared analysis of observed and expected male *Tigriopus californicus* mortality.

	SOURCE		TOTAL	MALE MORTALITY			
Acclimation Temperature	Acclimation Salinity	Stress Temperature	MORTALITY	Observed	Expected	$\frac{(O-E)^2}{E}$	Summation
10° C.	10 o/oo	31° C.	27.67%	48.08%	26.34%	17.94[3]	
10	35	33	16.83	70.97	31.22	50.61[3]	
10	50	33	7.35	55.00	25.74	33.26[3]	
10	50	34	10.53	57.14	28.80	28.36[3]	
10	70	34	34.71	33.33	24.53	3.16NS	
18	10	30	13.07	58.33	23.60	51.11[3]	
18	10	31	29.26	61.11	30.83	29.74[3]	
18	35	33	37.08	31.33	16.67	12.89[3]	
18	50	34	12.38	46.67	34.59	4.22[1]	
18	70	34	38.53	57.45	36.36	12.23[3]	
25	10	30	17.40	40.00	37.50	0.17NS	
25	10	31	32.61	42.86	26.67	9.83[2]	
25	25	32	12.76	72.72	15.89	203.25[3]	
25	35	33	25.76	52.50	28.29	20.72[3]	
25	50	33	14.14	61.11	22.64	65.37[3]	
25	50	34	20.11	54.55	28.04	25.06[3]	
25	70	33	10.54	35.71	34.56	0.04NS	
25	70	34	39.86	64.52	33.77	28.00[3]	
							595.96[3] (df=17)
10	25	33	57.87	36.31	28.72	2.01NS	
10	35	34	70.13	38.59	31.46	1.62NS	
10	50	35	53.17	31.76	30.38	.06NS	
10	70	35	51.39	31.32	26.42	.91NS	
18	25	33	56.32	28.23	23.11	1.13NS	
18	35	34	57.83	31.34	31.14	0.0012NS	
18	50	35	79.99	20.75	19.70	.06NS	
18	70	35	65.98	35.85	27.95	2.23NS	

Table VIII continued.

SOURCE			TOTAL MORTALITY	MALE MORTALITY		$\frac{(O-E)^2}{E}$	Summation
Acclimation Temperature	Acclimation Salinity	Stress Temperature		Observed	Expected		
25° C.	25 o/oo	33° C.	60.23%	43.70%	38.57%	0.68NS	
25	35	34	59.38	36.36	31.58	0.72NS	
25	50	35	66.56	38.37	28.91	3.10NS	
25	70	35	64.15	40.63	27.35	6.43[1]	
							18.95NS (df=11)
							614.91[3] (df=29)

[1] $p < 0.05$

[2] $p < 0.01$

[3] $p < 0.001$

Table IX Median survival times (LD_{50}) for *Tigriopus californicus* held in twelve salinities at two temperatures.

	Median Survival Time (LD_{50})			
	8° C.		26° C.	
Salinity	Males	Females	Males	Females
DW	0.00 days	0.00 days	0.00 days	0.00 days
5 o/oo	2.36	9.50	0.76	0.82
10	4.50	10+	2.44	3.64
20	8.50	10+	3.00	6.80
30	10+	10+	6.34	10+
50	10+	10+	6.32	10+
70	10+	10+	6.40	10+
80	10+	10+	2.82	7.57
90	10+	10+	2.23	4.19
100	5.84	10+	2.00	4.09
110	2.26	7.88	1.39	2.43
120	0.00	0.00	0.00	0.00

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