# THERMAL RESISTANCE AND ACCLIMATION AT VARIOUS SALINITIES IN THE SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS* LACEPEDE)


Prepared by

HERBERT BENTON SIMMONS

Department of Wildlife Science





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## ABSTRACT

Thermal Resistance and Acclimation at Various Salinities
in the Sheepshead Minnow
(*Cyprinodon variegatus* Lacépède). (May 1971)
Herbert Benton Simmons, B.S., Texas A&M University;
Directed by: Dr. Kirk Strawn

*Cyprinodon variegatus* collected in November 1969 on Galveston Island, Texas at 19.2 C and 2.7 $^{0}/00$ salinity were acclimated at 30 C and 1, 10, 20, and 30 $^{0}/00$ salinity and subjected to heat death at 40.8 and 41.4 C at their acclimation salinity. Samples were killed daily for 26, 29-30, 21, and 11 days respectively at 1, 10, 20, and 30 $^{0}/00$. At the end of the experiment, survival times of fish being acclimated at 1, 20, and 30 $^{0}/00$ were still increasing. These results are at variance with the assumption, usually used in high temperature experiments with fishes, that acclimation is complete in 3 days. At 10 $^{0}/00$ the highest level of acclimation recorded was attained within 14 and 15 days at a kill temperature of 41.4 and 40.8 C respectively. Fish were the most resistant to heat at 10 and 20 and the least at 1 and 30 $^{0}/00$. Survival times fluctuated among days. Sex and length were a factor in survival time only at 1 $^{0}/00$ salinity (sex only at 40.8 C). Long and male fish had the longest survival times.

## ACKNOWLEDGEMENTS

I would like to express my appreciation to the following people for their assistance in the completion of this thesis. First I would like to thank Dr. Kirk Strawn, Committee Chairman, for his help in the planning of the experiment and for his advice in the preparation of the manuscript. I would also like to thank Dr. Dave Aldrich, Committee member, for his assistance and encouragement in the laboratory and in editing the manuscript. Thanks also go to Dr. R. J. Freund, Committee member, for his explanation and direction in the statistical analysis of the data. Appreciation is extended to Mr. Brad Lisenbe, Texas A&M University, who programed the data for the computer and to Mr. R. R. Proctor, Bureau of Commerical Fisheries, for the water analysis. I would also like to thank my wife, Janet, for her assistance in the laboratory and for her understanding. To her and our son Michael, this thesis is dedicated.

These experiments were done in space provided by the Texas A&M University Marine Laboratory, Galveston, Texas. The study was financed by the National Science Foundation Sea Grant Program, Institutional Grant GH-101, made to Texas A&M University and by the Texas Agricultural Experiment Station Project 4011.

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# INTRODUCTION

Interest in the culture of both marine and fresh-water fishes has increased markedly in the past few years. The stocking of waters with marine fish for culture frequently involves taking fish from one salinity and temperature and putting them at a different combination of salinity and temperature. The ability of fish to withstand these changes depends, at least in part, on the rate at which their metabolic processes can adjust to new levels. While very little work has been done on the acclimation rates of fishes, it is known that the rate varies among species. This rate needs to be known before efficient experiments can be designed to determine the safe limits of temperature-salinity combinations when stocking waters with fishes. *Cyprinodon variegatus* is one of the most resistant of fishes to high temperature. It is commonly used for bait and can be raised in fish ponds on the coast for bait use in both marine waters and in the highly mineralized waters found in western Texas. The purpose of this study was to determine the acclimation rates for *Cyprinodon*

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The citations in this thesis follow the style of the *Transactions of the American Fisheries Society*.

*variegatus* to 30 C at various salinities. Salinity was used as a variable because it was suspected that salinity would influence the acclimation rate.

# REVIEW OF THE LITERATURE

Two subspecies of the sheepshead minnow are recognized along the Atlantic and Gulf coasts of the United States (Hubbs, 1936). *Cyprinodon variegatus ovinus* which is found from Massachusetts to Virginia and *Cyprinodon variegatus variegatus* found from North Carolina to near Brownsville, Texas.

*C. variegatus* is a coastal species, being found in a wide range of salinities. Kilby (1955) noted, in work done along the Gulf coast of peninsular Florida, that *C. variegatus* was found in salinities ranging from 0.0 to 35.6 $^{0}/00$ with a preference being shown for salinities less than 20 $^{0}/00$. He also noted a preference for areas where submerged vegetation was either sparse or absent. Simpson and Gunter (1956) reported finding *C. variegatus* alive in drying ponds having a salinity of 142.4 ppt. They state that this is probably close to the limit of tolerance as one seine haul in the Laguna Madre, where the salinity was 147.0 ppt., produced only dead fish.

*C. variegatus* exhibits sexual dimorphism and the males have been found to be territorial. Raney et al. (1953) found that each male establishes a territory up to 1½ to 2 ft in diameter. No other fish is allowed in this territory until a female enters the nest and remains despite attacks from the male. This indicates that she is ready to spawn. The male continues to spawn as long as females move into his territory. Spawning seems to occur throughout most of the year, both individually and collectively (Hildebrand, 1919).

Kinne (1960) found that in the desert pupfish, Cyprinodon macularius, the temperature needed for optimum growth decreased with an increase in age of the fish. He found that the lethal temperature for fertilized eggs is a function of salinity and oxygen (Kinne and Kinne, 1962). Strawn and Dunn (1967) found C. variegatus to be the most resistant to heat death of any of the marsh fishes used in their study.

Doudoroff (1942) and Brett (1944) found that fish transferred to a higher temperature gained resistance to this higher temperature at a faster rate than they lost this resistance when transferred back to a lower temperature. Doudoroff found that the greenfish, Girella nigricans (Ayres), gained heat resistance rapidly when fish acclimated to 14 C were transferred to 20 C. Acclimation to the new temperature was apparently complete after about one day. Brett, working with Ictalurus nebulosus, transferred fish acclimated to 20 C to water at 28 C. Survival time for these fish after 24 hours of acclimation was equivalent to survival time for bullheads acclimated to 28 C. In both the studies it was found that when fish acclimated to the higher temperatures were transferred to the lower ones, at least 20 days were required for complete acclimation to take place. Brett (1956) states that the rate of acclimation appears to be governed by the rate of metabolism. This is pointed out in his work on goldfish (Brett, 1946) where equal changes in temperatures at various levels resulted in different lengths of time required for acclimation. His comparison of goldfish and brown bullhead data show that under the same temperature conditions, the bullhead reaches complete acclimation in one-third the time it takes for goldfish. Allanson and Noble (1964) working with *Tilapia mossambica* (Peters) found that the level of thermal resistance to 30 C reached its peak at 16 to 24 hours of acclimation. They believe that the loss of resistance after 24 hours could be due to a complex response involving compensation to a higher temperature coupled with a response to a change in temperature.

## METHODS AND MATERIALS

On Nov. 5, 1969, approximately 4000 _C. variegatus_ were seined and taken to the Texas A&M Marine Laboratory at Galveston, Texas. The site of capture was a pond approximately 2 acres in size. The pond is located just north of the west end of the Galveston Island sea wall. Transfer of the fish from the field to the laboratory was accomplished using aerated tanks mounted on a pickup truck. The tanks were filled with water from the site of capture. This water was at 19.2 C and the salinity was 2.7 $^{0}/_{00}$.

On reaching the laboratory the fish were placed in a holding tank containing water of approximately the same salinity and temperature (21.3 C; 3.8 $^{0}/_{00}$) as at the site of capture. From the holding tank they were immediately counted out within 3 hours (by 1630) into 20 acclimation tanks (200 per tank) containing water at 30 C. Water temperature was controlled using contact thermometers in conjunction with electrical relays. This system provided an accuracy of $\pm$ 0.1 C. The water in each tank was at one of four salinities. Acclimation tanks No. 1-5 were maintained at 1 $^{0}/_{00}$, No. 6-10 at 10 $^{0}/_{00}$, No. 7-15 at 20 $^{0}/_{00}$, and No. 16-20 at 30 $^{0}/_{00}$ salinity. The acclimation tanks were made of plywood, insulated with foam plastic, and covered with a polyester resin. Each tank contained approximately 70 gallons of natural seawater diluted with Galveston city water to required salinities and evaporative losses were replaced with distilled water. The water in each tank was filtered using a submerged gravel filter covered with a mixture of crushed oyster shell and coarse sand. Compressed air was supplied through four air stones which stirred the water and provided oxygen. Continuous light was provided using a "cool white" fluorescent tube suspended about 1 ft above the water.

The lethal-temperature baths were made of plywood coated with polyester resin except for the front and lid which are made of plexiglass. Each bath contained approximately 6 gallons of natural sea water diluted to experimental salinities with distilled water. The regulation of temperature, oxygen, and the filtering of water was accomplished in the same manner as in the acclimation tanks.

On Nov. 6 at approximately 0900, 20 fish from each salinity (four from each acclimation tank) were placed at 40.8 C in four lethal-hot-water baths of 3, 10, 20, and 30 $^{0}/_{00}$ salinity. All fish were killed at the same salinity at which they were acclimated. Beginning on

Nov. 7, 10 fish from each salinity (two from each acclimation tank) were killed at 41.4 C in addition to the fish killed at 40.8 C. The two lethal temperatures were used to see if acclimation rates had a similar pattern at different temperatures.

After the fish were placed in the hot water baths the time to death for each individual was recorded. Lack of movement of the operculum and branchiostegel membrane was used as the criteria for death. The fish were then removed and the standard length and sex recorded.

The above procedure was repeated each day at approximately the same time of day until all of the fish were used.

The fish were fed once a day at approximately 0800 and temperature and salinity of each tank was checked each day prior to beginning the experiment. Water samples from each tank were taken on the first day and then once a week to determine nitrogen concentrations (Solorzano, 1969) in the water. On three different occasions the temperature in one of the acclimation tanks deviated from 30 C due to equipment breakdown. In each case there was no sample taken from the tank on that day and the proper temperature was restored as quickly as possible. After the first week fish in some of the acclimation tanks became too few in number to catch efficiently. When this occurred the tank was drained and the remaining fish placed in one of the remaining acclimation tanks of the same salinity.

The data obtained from the experiments was analyzed using the IBM 360 computer at Texas A&M University. This analysis consisted of a regression line for each temperature-salinity combination followed by an analysis of variance between kill temperatures and between salinities. A regression analysis between survival time and factors such as sex, length, and location in the kill tank at the time of death was also computed. Five percent significance levels were used except where noted.

# RESULTS

## Condition of Fish

The fish used in this experiment became accustomed to tank life quickly. Good feeding activity was noticed on the fourth day of acclimation. This feeding activity became stronger on the fifth day when the fish were changed from a granular (Purina Trout Chow) to a flake food diet (Tetra Min Tropical Fish Food). All significant mortality occurred during the first 3 days of acclimation. This mortality was 67.5 % at 30 $^{0}/_{00}$, 37.7 % at 20 $^{0}/_{00}$, 23.4 % at 1 $^{0}/_{00}$, and 9.8 % at 10 $^{0}/_{00}$ salinity. The duration of the experiments at each salinity level was inversely related to mortality in the acclimation tanks. Establishment of territories by males was noticed after about 2 weeks and reproduction occurred. Large numbers of juveniles, approximately 5 mm in total length, were present at a salinity of 1 $^{0}/_{00}$ when acclimation tanks 1 and 3 were drained November 27 and December 1 respectively. Water samples, taken once a week from each acclimation tank, were analyzed for $NH_3$ - $NH_4$ concentrations (Table 1). The highest concentration found was 0.318 ppm in tank 19 (30 $^{0}/_{00}$ salinity).

Table 1.--Concentrations of $NH_3$-$NH_4$ (ppm.) in acclimation tanks taken at weekly intervals

| Tank No. | Date | | | | | |
|---|---|---|---|---|---|---|
| | Nov. 5 | Nov. 12 | Nov. 19 | Nov. 26 | Dec. 3 | Dec. 5 |
| 1 | 0.006 | 0.039 | 0.019 | 0.036 | | |
| 2 | 0.003 | 0.044 | 0.104 | 0.039 | | |
| 3 | 0.014 | 0.056 | 0.026 | 0.028 | | |
| 4 | 0.016 | 0.045 | 0.029 | 0.046 | | |
| 5 | 0.005 | 0.038 | | 0.021 | | |
| 6 | 0.019 | 0.168 | 0.043 | 0.048 | 0.036 | |
| 7 | 0.064 | 0.218 | 0.073 | 0.141 | 0.028 | |
| 8 | 0.018 | 0.096 | 0.038 | 0.036 | 0.036 | |
| 9 | 0.021 | 0.077 | 0.022 | 0.039 | | 0.069 |
| 10 | 0.014 | 0.099 | 0.038 | 0.039 | 0.045 | |
| 11 | 0.020 | 0.093 | 0.120 | 0.288 | | |
| 12 | 0.016 | 0.111 | 0.102 | | | |
| 13 | 0.015 | 0.000 | 0.301 | | | |
| 14 | 0.004 | 0.017 | 0.056 | | | |
| 15 | 0.006 | 0.119 | 0.038 | | | |
| 16 | 0.004 | 0.096 | | | | |
| 17 | 0.012 | 0.141 | | | | |
| 18 | 0.015 | 0.060 | | | | |
| 19 | 0.036 | 0.318 | | | | |
| 20 | 0.020 | 0.075 | | | | |

Results of Test at 40.8 C

Samples of 20 fish from each salinity were killed once every 24 hours beginning on November 6 at approximately 0900. The number of fish killed at 40.8 C was 516 at 1 $^{0}/00$, 580 at 10 $^{0}/00$, 414 at 20 $^{0}/00$, and 221 at 30 $^{0}/00$ salinity. The first kill, on November 6, at 18 hours of acclimation resulted in mean death times of 19.6 min. at 1 $^{0}/00$, 16.2 min. at 10 $^{0}/00$, 34.9 min. at 20 $^{0}/00$, and 29.9 min. at 30 $^{0}/00$ salinity. The overall pattern of mean-survival times showed an increase at all salinities as acclimation to 30 C became more complete, (Figures 1-7 and Tables 2, 3). Daily oscillations in the mean-survival times are noticeable. These oscillations seem to occur in all data of this type and no one has been able to isolate the variable responsible. A regression equation of the form $\text{Log}_e y = B_0 + B_1 (1/x) + B_2 (1/x)^2$ was computed at each salinity for survival time (y) on acclimation time (x) (Table 4). This particular form of equation readily provides for an asymptote, that is, a maximum survival time regardless of the length of acclimation. This is obtained through the y-intercept, $B_0$, which is the value of $\log_e y$ if the independent variables $(\frac{1}{x}, \frac{1}{x}^2)$ are zero which occurs

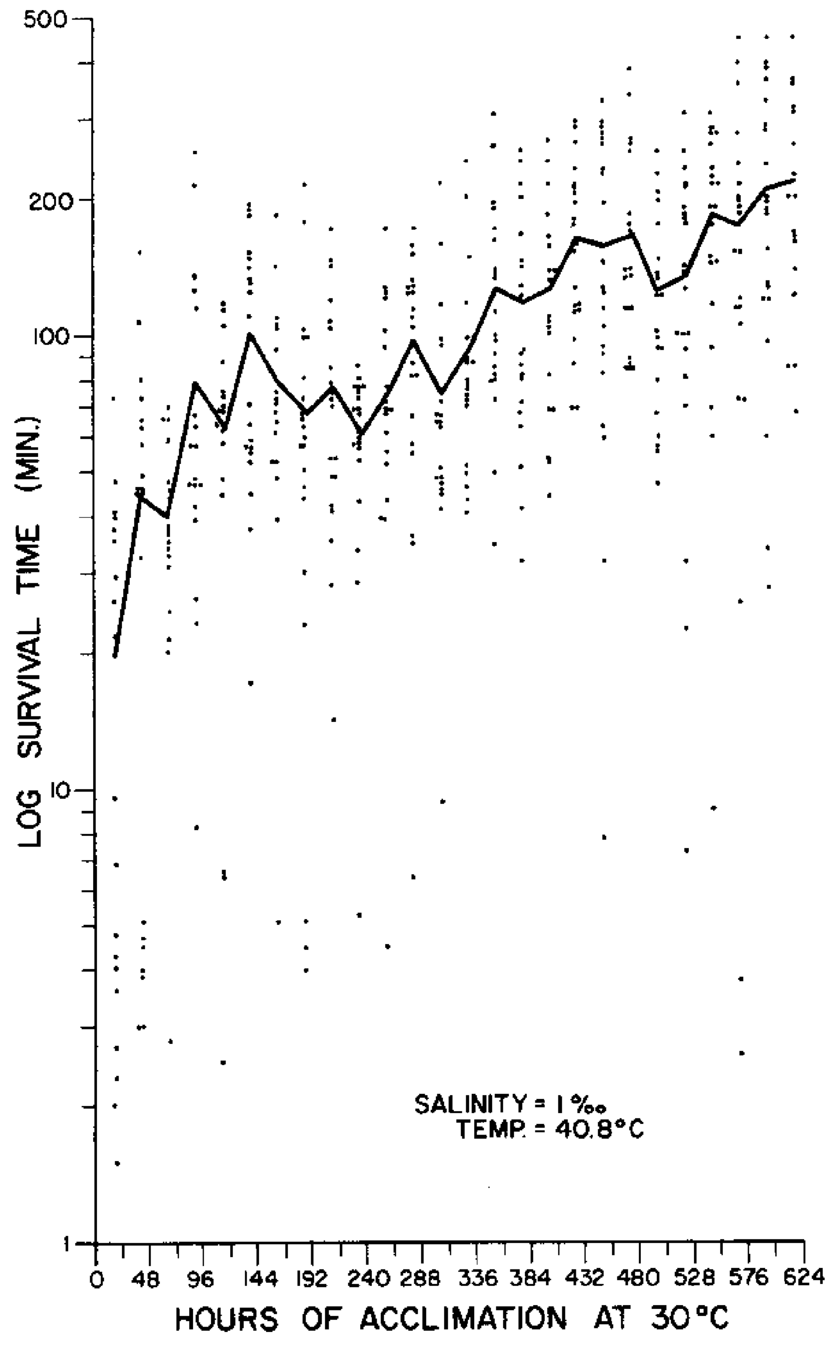


Figure 1. Daily change of survival times for *Cyprinodon variegatus* captured at 19.2 C, 2.7 $^{0}/_{00}$ salinity; acclimated at 30 C, 1 $^{0}/_{00}$ salinity; and killed at 40.8 C, 1 $^{0}/_{00}$ salinity. Individual and mean survival times are represented by dots and a solid line respectively.

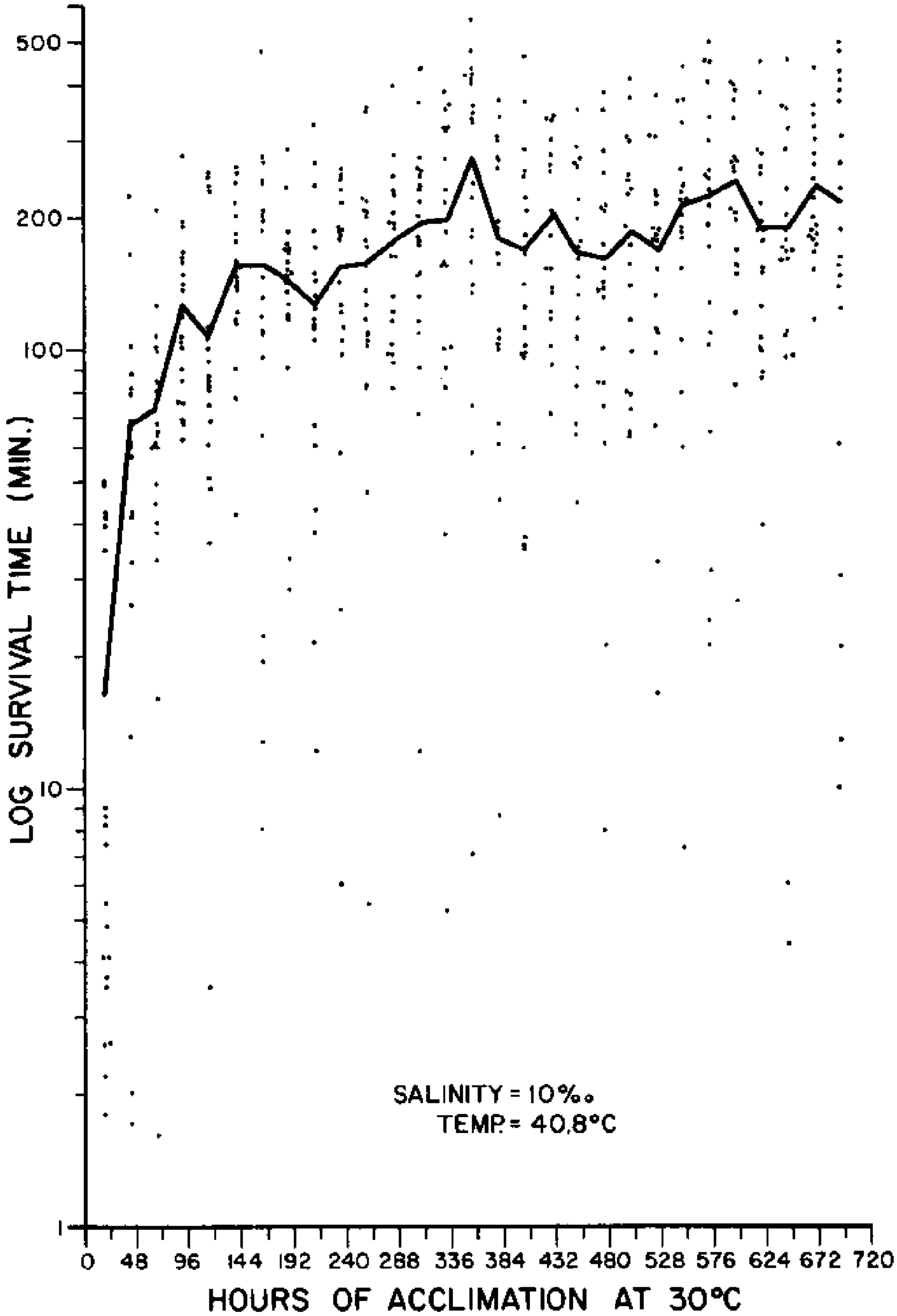


Figure 2. Daily change of survival times for *Cyprinodon variegatus* captured at 19.2 C, 2.7 $^{0}/00$ salinity; acclimated at 30 C, 10 $^{0}/00$ salinity; and killed at 40.8 C, 10 $^{0}/00$ salinity. Individual and mean survival times are represented by dots and a solid line respectively.

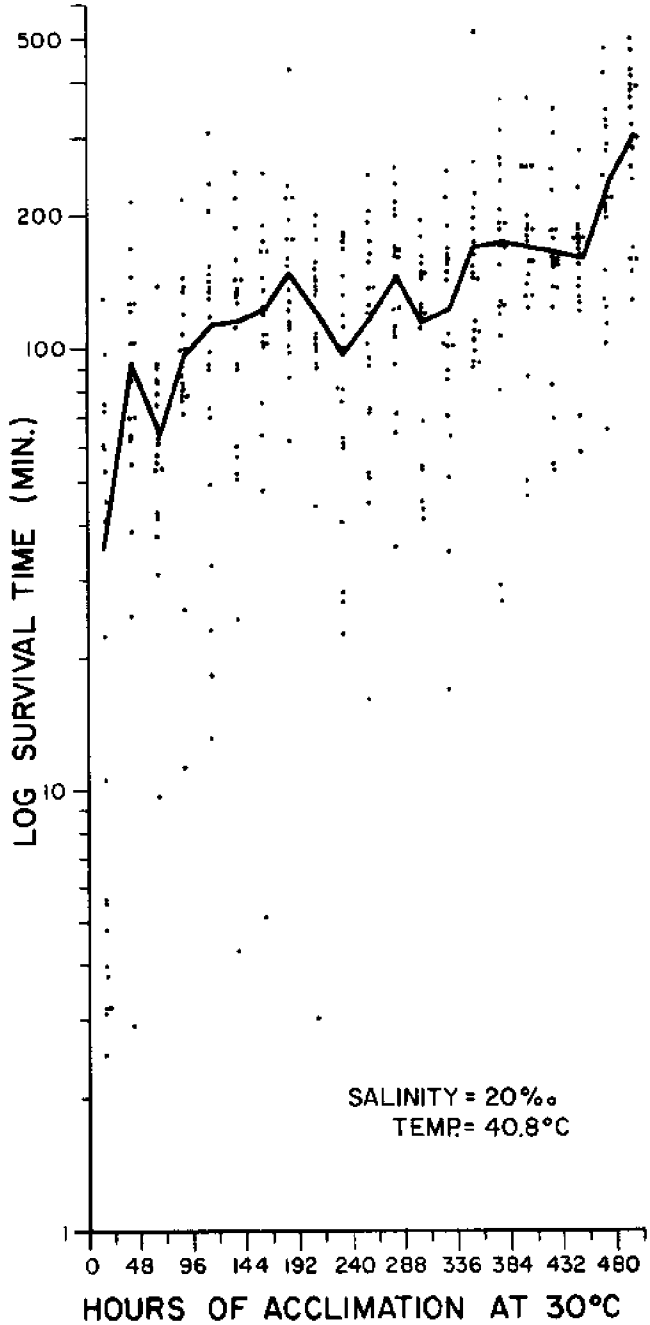


Figure 3. Daily change of survival times for *Cyprinodon variegatus* captured at 19.2 C, 2.7 $^0/00$ salinity; acclimated at 30 C, 20 $^0/00$ salinity; and killed at 40.8 C, 20 $^0/00$ salinity. Individual and mean survival times are represented by dots and a solid line respectively.

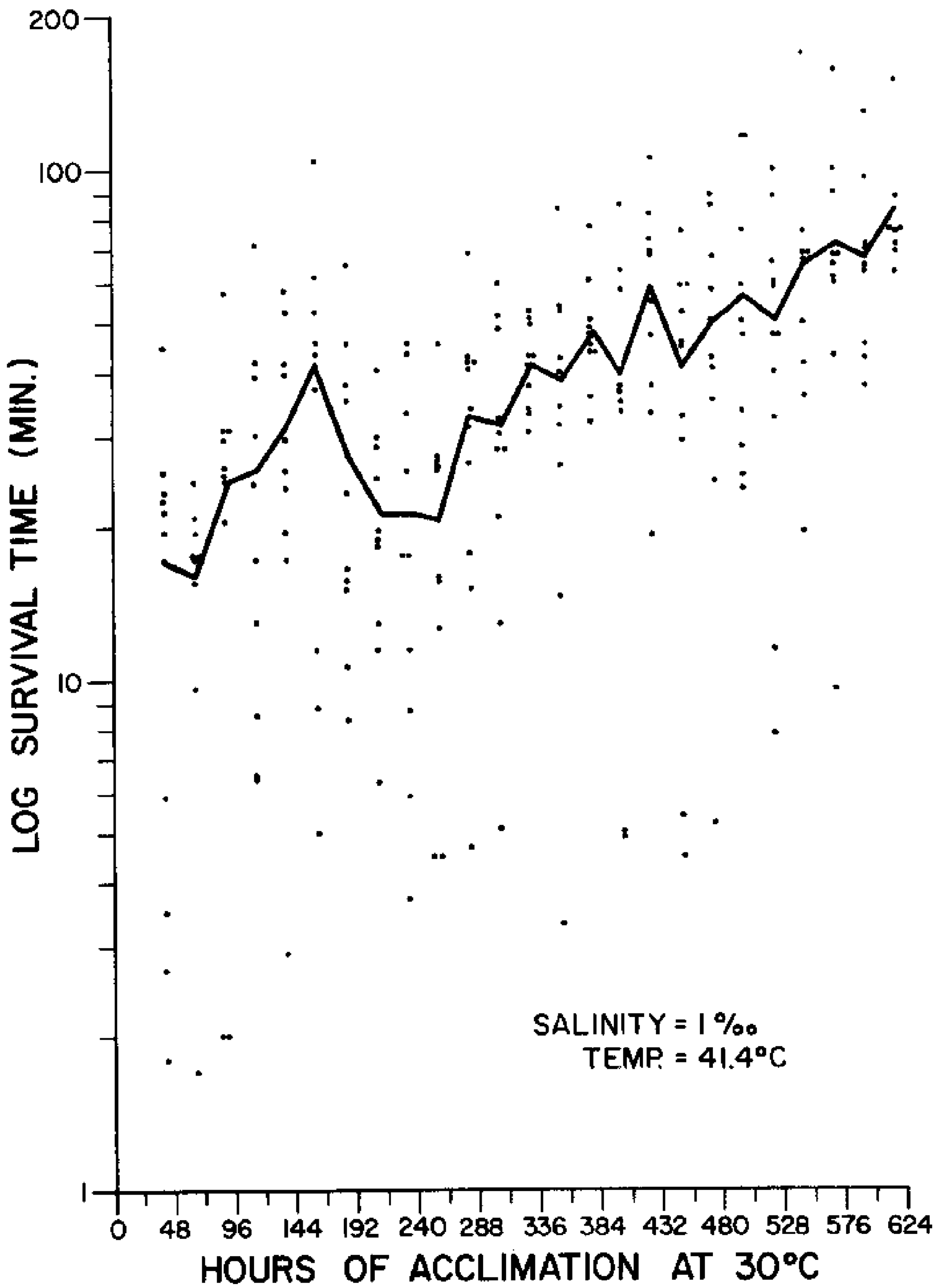


Figure 4. Daily change of survival times for *Cyprinodon variegatus* captured at 19.2 C, 2.7 $^{0}/00$ salinity; acclimated at 30 C, 1 $^{0}/00$ salinity; and killed at 41.4 C, 1 $^{0}/00$ salinity. Individual and mean survival times are represented by dots and a solid line respectively.

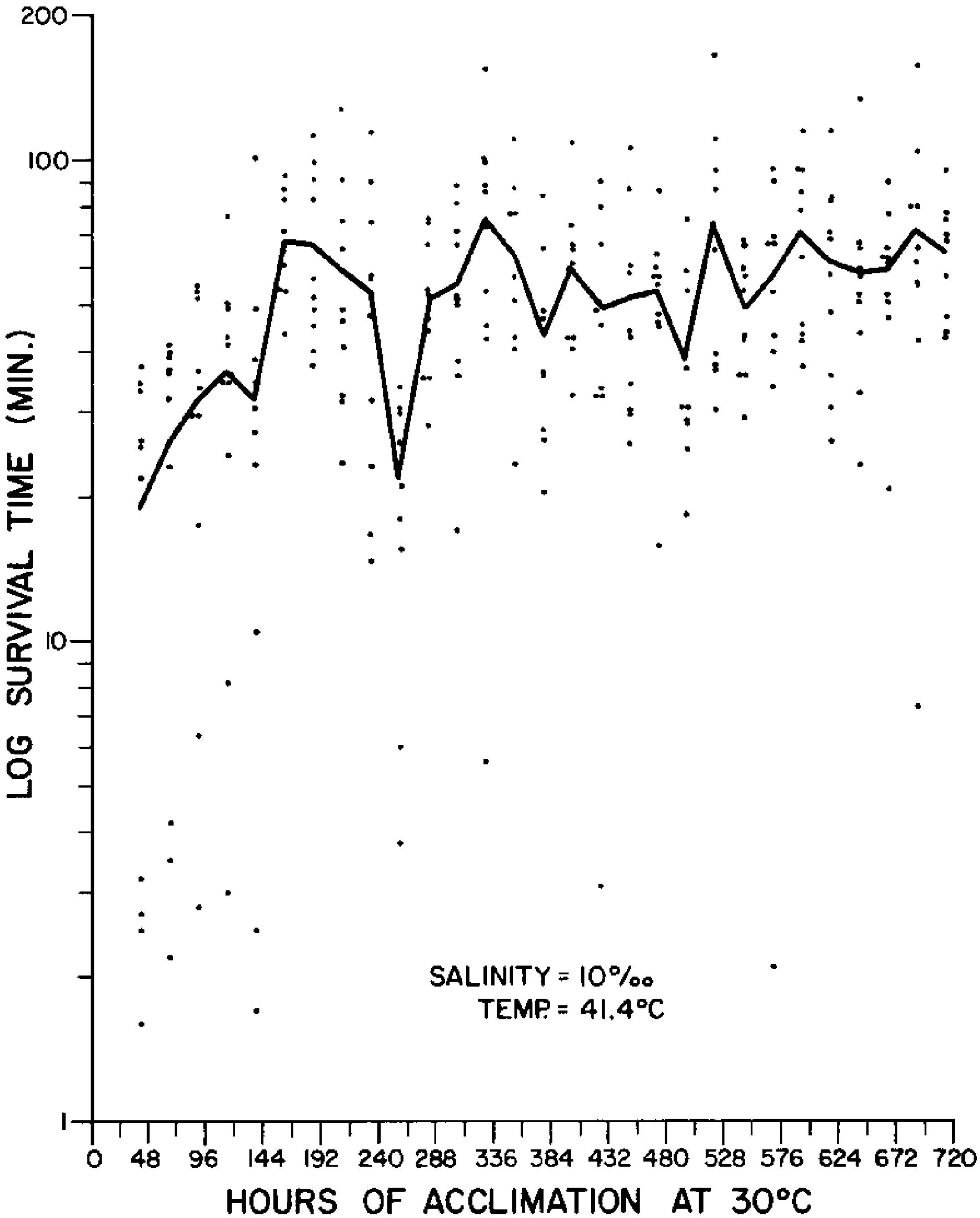


Figure 5. Daily change of survival times for *Cyprinodon variegatus* captured at 19.2 C, 2.7 $^0/00$ salinity; acclimated at 30 C, 10 $^0/00$ salinity; and killed at 41.4 C, 10 $^0/00$ salinity. Individual and mean survival times are represented by dots and a solid line respectively.

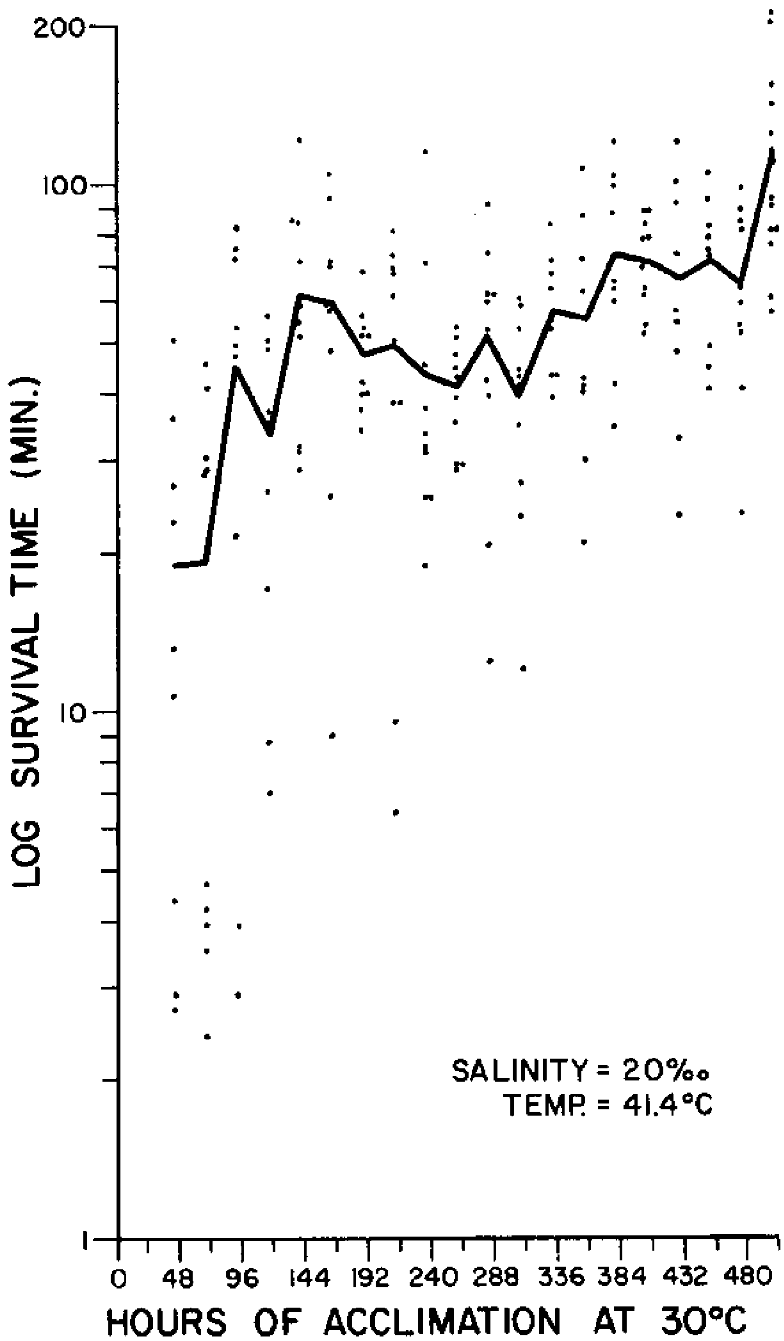


Figure 6. Daily change of survival times for *Cyprinodon variegatus* captured at 19.2 C, 2.7 $^{0}/00$ salinity; acclimated at 30 C, 20 $^{0}/00$ salinity; and killed at 41.4 C, 20 $^{0}/00$ salinity. Individual and mean survival times are represented by dots and a solid line respectively.

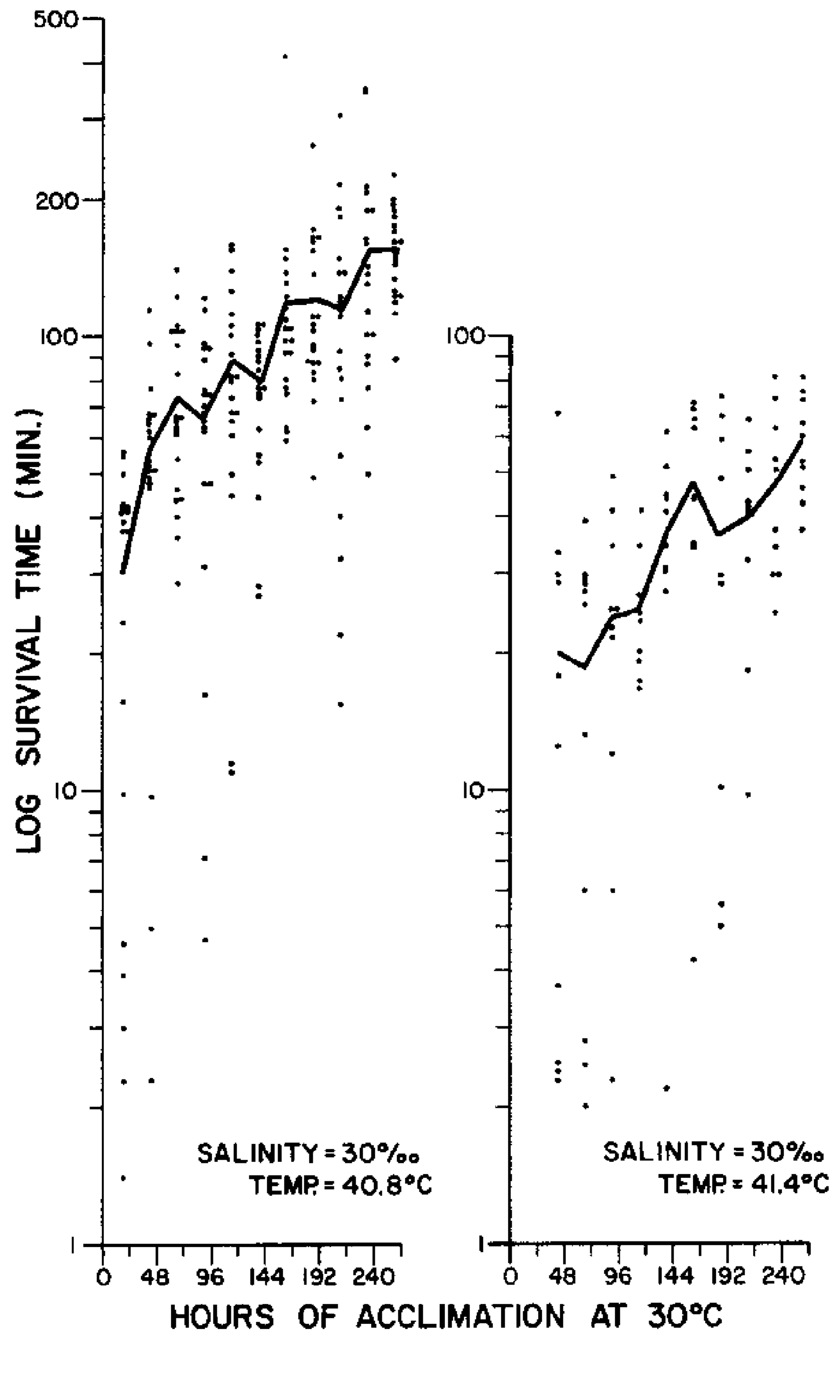


Figure 7. Daily change of survival times for *Cyprinodon variegatus* captured at 19.2 C, 2.7 $^0/00$ salinity; acclimated at 30 C, 30 $^0/00$ salinity; and killed at 40.8 C and 41.4 C, 30 $^0/00$ salinity. Individual and mean survival times are represented by dots and solid lines respectively.

Table 2.--Daily mean-survival times at each salinity for fish killed at 40.8 C (kill on day one was at 18 hours of acclimation and subsequent kills at 24 hour intervals)

| Day | Salinity (⁰/oo) 1 | 10 | 20 | 30 |
|---|---|---|---|---|
| 1 | 19.6 | 16.2 | 34.9 | 29.9 |
| 2 | 44.2 | 66.2 | 92.5 | 54.4 |
| 3 | 39.7 | 73.5 | 63.4 | 73.0 |
| 4 | 78.7 | 125.9 | 96.4 | 65.8 |
| 5 | 62.3 | 107.6 | 112.7 | 88.1 |
| 6 | 100.7 | 154.6 | 113.8 | 79.6 |
| 7 | 79.5 | 155.7 | 120.8 | 117.2 |
| 8 | 67.5 | 146.1 | 146.8 | 119.3 |
| 9 | 76.8 | 127.2 | 120.7 | 114.2 |
| 10 | 60.1 | 152.7 | 96.9 | 153.0 |
| 11 | 76.7 | 158.3 | 118.1 | 154.0 |
| 12 | 97.3 | 175.2 | 145.1 | |
| 13 | 75.1 | 196.4 | 114.0 | |
| 14 | 91.9 | 196.1 | 120.2 | |
| 15 | 128.4 | 273.9 | 167.0 | |
| 16 | 118.3 | 178.7 | 171.2 | |
| 17 | 127.9 | 166.4 | 169.6 | |
| 18 | 162.7 | 199.6 | 162.6 | |
| 19 | 158.7 | 167.8 | 158.6 | |
| 20 | 167.3 | 159.5 | 229.3 | |
| 21 | 123.5 | 186.7 | 303.2 | |
| 22 | 137.1 | 169.0 | | |
| 23 | 181.1 | 210.2 | | |
| 24 | 171.7 | 222.3 | | |
| 25 | 209.9 | 236.7 | | |
| 26 | 216.1 | 188.2 | | |
| 27 | | 189.1 | | |
| 28 | | 233.5 | | |
| 29 | | 217.5 | | |
| 30 | | | | |

Table 3.--Daily mean-survival times at each salinity for fish killed at 41.4 C (first kill was on day two at 42 hours of acclimation and subsequent kills at 24 hour intervals

| Day | Salinity (0/00) 1 | 10 | 20 | 30 |
|---|---|---|---|---|
| 1 | | | | |
| 2 | 17.1 | 18.8 | 18.9 | 20.1 |
| 3 | 16.1 | 25.9 | 19.3 | 18.7 |
| 4 | 24.8 | 31.6 | 45.3 | 24.0 |
| 5 | 25.8 | 36.6 | 33.6 | 24.9 |
| 6 | 31.1 | 31.9 | 62.0 | 36.7 |
| 7 | 41.0 | 67.9 | 59.6 | 47.5 |
| 8 | 27.3 | 66.6 | 47.5 | 36.3 |
| 9 | 21.2 | 58.2 | 49.4 | 39.9 |
| 10 | 21.3 | 52.6 | 43.5 | 47.6 |
| 11 | 20.5 | 21.8 | 40.2 | 58.3 |
| 12 | 33.0 | 51.4 | 51.5 | |
| 13 | 31.7 | 55.2 | 39.7 | |
| 14 | 41.4 | 74.8 | 57.4 | |
| 15 | 38.2 | 63.2 | 55.7 | |
| 16 | 48.3 | 43.5 | 73.5 | |
| 17 | 39.7 | 59.5 | 72.0 | |
| 18 | 58.6 | 48.8 | 66.8 | |
| 19 | 40.6 | 51.8 | 71.3 | |
| 20 | 49.4 | 54.5 | 64.3 | |
| 21 | 56.2 | 38.7 | 113.6 | |
| 22 | 50.5 | 73.8 | | |
| 23 | 65.7 | 49.0 | | |
| 24 | 71.2 | 56.5 | | |
| 25 | 67.7 | 49.0 | | |
| 26 | 82.9 | 61.6 | | |
| 27 | | 58.4 | | |
| 28 | | 59.0 | | |
| 29 | | 70.8 | | |
| 30 | | 64.0 | | |

Table 4.--Values for the regression equation $\mathrm{Log}_e y = B_0 + B_1(\frac{1}{x}) + B_2(\frac{1}{x})^2$ at 40.8 C for each salinity

| Salinity ($^0/00$) | $B_0$ | $B_1$ | $B_2$ | d.f. for t |
|---|---|---|---|---|
| 1 | 2.142 | -49.813 (t=-9.585)** | 551.370 (t=5.811)** | 513 |
| 10 | 2.236 | -24.018 (t=-16.710)** | --- | 577 |
| 20 | 2.171 | -17.902 (t=-13.386)** | --- | 411 |
| 30 | 2.193 | -32.936 (t=-5.209)** | 294.801 (t=2.786)** | 218 |

$B_2$ was eliminated from equation at 10 and 20 $^0/00$ due to lack of significance.

** = Significance at the 0.01 level

only when $x = \infty$. At salinities of 10 and 20 $^{0}/_{00}$ the inclusion of the quadratic term in the regression equation resulted in no significant improvement in the regression. This result shows that fish at 10 and 20 $^{0}/_{00}$ salinity had a faster rate of acclimation to 30 C than did fish at 1 and 30 $^{0}/_{00}$ salinity. An analysis of variance on the regression lines at each salinity was computed. F values for the corrected regression equations showed a highly significant regression at all salinities.

A combined analysis of variance of regression lines within each kill temperature was computed (Table 5). This "step down" analysis first allowed all data at 40.8 C to be used in one regression. The next step in the analysis was to allow $B_0$ to vary between salinities. This model specifies that the rate of response of y to a given change in x-values is the same but the level of y may differ among groups of data (different temperature-salinity combinations). An F Test showed that at 40.8 C this gave a highly significant improvement over the single regression ($F = 29.173$; $df = 3,1725$). The third step was to allow $B_0$, $B_1$, and $B_2$ all to vary at each salinity, that is, both the rate and level of response of y may differ among groups of data. An F test showed a highly

Table 5.--Combined analysis of variance of data collected at 40.8 C at salinities of 1, 10, 20, 30 $^{0}/00$

| Source | DOF | SS | MS | F-Value |
|---|---|---|---|---|
| Total | 1703 | 319.113 | .184 | |
| One Reg. | 2 | 98.617 | 49.336 | 386.735** |
| Resid. (1) | 1728 | 220.441 | .127 | |
| Add Intercept | 3 | 10.644 | 3.548 | 29.173** |
| Resid. (2) | 1725 | 209.797 | .122 | |
| Add Coeff. | 6 | 3.449 | .575 | 4.789** |
| Resid. (3) | 1719 | 206.348 | .120 | |

** = Significance at the 0.01 level

significant improvement ($\alpha$ = 0.01) over step number two ($F$ = 4.789, df = 6,1719). The relatively small reduction in residual mean square (0.122 to 0.120) indicates that this improvement may not be very meaningful. Alpha values, mean asymptotic values in minutes, (antilog of $B_0$) for the four salinities show that survival times at 10 and 20 $^{0}/_{00}$ salinity were higher than at 1 and 30 $^{0}/_{00}$ (Table 6).

Table 6.--Estimated asymptote (maximum survival time) in minutes

| | | Salinity ($^{0}/_{00}$) | | | |
|---|---|---|---|---|---|
| | | 1 | 10 | 20 | 30 |
| Lethal | 40.8° C | 121.5 | 184.1 | 179.3 | 168.8 |
| Temperature | 41.4° C | 54.5 | 72.2 | 96.1 | 70.7 |

Results of Tests at 41.4 C

Samples of 10 fish from each salinity were killed once every 24 hours beginning on November 7 at approximately 0900. The number of fish killed at 41.4 C was 246 at 1 $^{0}/_{00}$, 290 at 10 $^{0}/_{00}$, 202 at 20 $^{0}/_{00}$, and 101 at 30 $^{0}/_{00}$ salinity. The first kill, on November 7, at 42 hours of acclimation resulted in mean data times of 17.1 min. at 1 $^{0}/_{00}$, 18.8 min. at 10 $^{0}/_{00}$, 18.9 min. at 20 $^{0}/_{00}$, and 20.1 min. at 30 $^{0}/_{00}$ salinity. The mean survival times oscillated from day to day as they did for fish killed at 40.8 C. However, no agreement was found either among salinities or between kill temperatures, in the increase or decrease of mean survival times on a particular day. (Tables 2, 3). The same regression equation that was employed for fish killed at 40.8 was computed for fish killed at 41.4 C. Only at 10 $^{0}/_{00}$ salinity did the inclusion of the quadratic term in the equation result in no improvement in the regression, showing these fish to have a faster rate of acclimation than fish at the other salinities (Table 7). Again, a combined analysis of variance between salinities showed that allowing $B_0$ to vary gave a highly significant improvement over the single regression ($F = 14.310$; df = 3,833). Allowing all B's to vary, however, did not improve the mean square significantly (Table 8). Alpha values for the four salinities show that survival times at 10 and 20 $^{0}/_{00}$ salinity were again higher than at 1 and 30 $^{0}/_{00}$ (Table 6).

Table 7.--Values for the regression equation
$\mathrm{Log}_e y = B_0 + B_1(\frac{1}{x}) + B_2(\frac{1}{x})^2$ at 41.4 C for each salinity

| Salinity ($^0/00$) | $B_0$ | $B_1$ | $B_2$ | d.f. for t |
|---|---|---|---|---|
| 1 | 1.790 | -86.409 (t=-5.706)** | 2400.249 (t=3.707)** | 243 |
| 10 | 1.772 | -33.107 (t=-8.519)** | --- | 287 |
| 20 | 1.959 | -68.635 (t=-4.875)** | 1275.308 (t=2.154)* | 199 |
| 30 | 1.989 | -90.699 (t=-3.222)** | 2118.636 (t=2.054)* | 98 |

$B_2$ was eliminated from equation at 10 $^0/00$ due to lack of significance.

* = Significance at the 0.05 level.

** = Significance at the 0.01 level.

Table 8.--Combined analysis of variance of data collected at 41.4 C at salinities of 1, 10, 20, 30 $^{0}/_{00}$

| Source | DOF | SS | MS | F-Value |
|---|---|---|---|---|
| Total | 838 | 122.576 | 0.146 | |
| One Regression | 2 | 29.723 | 14.862 | 133.808** |
| Resid. (1) | 836 | 92.853 | 0.111 | |
| Add Intercept | 3 | 4.551 | 1.517 | 14.309** |
| Resid. (2) | 833 | 88.302 | 0.106 | |
| Add coeff. | 6 | 1.015 | 0.169 | 1.603 |
| Resid. (3) | 827 | 87.287 | 0.105 | |

** Significance at the 0.01 level

## Analysis of Variance Between Kill Temperatures

A combined analysis of variance between lethal temperatures, was computed. The second step of the analysis showed that at all salinities separate parallel lines were significantly better ($\alpha = 0.01$) than one regression line for all salinities. The third step allowed freedom of all variables (B's). The results showed that the regression lines were not parallel for data at 20 $^0/00$ salinity. Significance was at the 0.01 level. Data at 1 $^0/00$ was significant at the 0.05 level but the reduction in the mean square was only 0.001.

## Results of Additional Factors

The above discussed equations for the analysis of survival times were modified to include possible effects due to sex, length (Table 9). The modified equation took the form of

$$\mathrm{Log}_e y = B_0 + B_1(\frac{1}{x_1}) + B_2(\frac{1}{x_1})^2 + B_3X_2 + B_4X_3$$

where $X_2$ was the length of the fish and $X_3$ was the sex ($X_3$ = 1 for female and 2 for male). It was noticed, during the experiment, that while some fish floated at the time of death, others sank to the bottom of the

Table 9.--Results of regression analysis between survival time and the length and sex of the fish at four salinities

| | | 1 0/00 | 10 0/00 | 20 0/00 | 30 0/00 |
|---|---|---|---|---|---|
| 40.8°C | (Length) | +** | + | - | - |
| | (Sex) | +** | + | + | - |
| 41.4°C | (Length) | +* | - | - | - |
| | (Sex) | + | + | + | - |

Length;

+ = Longer fish had longer survival times

- = Longer fish had shorter survival times

Sex;

+ = Males had longer survival times than did females

- = Females had longer survival times than did males

* = Significance at the 0.05 level

** = Significance at the 0.01 level

tanks. The regression equation was modified to find out if this was significantly correlated with the survival time of the fish (Table 10). The equation took the form of

$$\text{Log}_e y = B_0 + B_1\left(\frac{1}{X_1}\right) + B_2\left(\frac{1}{X_1}\right)^2 + B_3X_4$$

where $X_4$ was the position of the fish in the tank at the time of death ($X_4$ = 1 for floating and 2 for bottom).

Sex was found to be a significant factor ($\alpha = 0.01$) only at 1 $^0/00$, 40.8 C. In this case males had longer survival times than did females. Length was found to be a significant factor at both lethal temperatures at 1 $^0/00$ salinity. In this case survival times increased with the length of the fish. At 40.8 C it was found that fish dying on the surface had longer resistance times (significant at 1, 10, 30 $^0/00$ salinity). At 41.4 C fish dying on the bottom had longer resistance times (significant only at 1 $^0/00$ salinity).

Table 10.--Results of regression analysis between survival time and the position of the fish in the lethal tank at the time of death at four salinities

| | 1 ‰ | 10 ‰ | 20 ‰ | 30 ‰ |
|---|---|---|---|---|
| 40.8°C | -* | -** | - | -** |
| 41.4°C | +** | + | + | + |

+ = Fish dying on the bottom of the tank had longer survival times

- = Fish dying on the top of the tank had longer survival times

* = Significance at the 0.05 level

** = Significance at the 0.01 level

# DISCUSSION

## Mortality

Most of the mortality which occurred in the acclimation tanks during the first 3 days of this study was apparently due to salinity shock. However, the fish, which were captured in water of 2.7 $^{0}/00$ salinity, had less mortality at 10 $^{0}/00$ salinity (9.8 % mortality) than fish placed in water of 1 $^{0}/00$ salinity (23.4 % mortality). Under temperature stress a concentration of dissolved salts higher than 1 $^{0}/00$ is of advantage to initial survival. These results agree with those reported by Strawn and Dunn (1967).

## Water Condition

The pH of the water in the acclimation tanks was not taken, however, the values for $NH_3$-$NH_4$ concentration were far below the lethal values for naturally buffered waters (Brown, 1957). Because natural sea water was used it is believed that the pH did not vary to the extremes required to bring about a detrimental effect due to ammonia concentrations.

## Oscillations in Survival Time

Although no statistical test for oscillations were performed, visual inspection definitely indicated the existance of this phenomenon.

The oscillations in the daily mean-survival times offer evidence of the complexity of determining the time required for complete acclimation. Since mean survival times for different samples of fish, killed on the same day at the same time, do not agree in their direction of change (higher or lower), it is hard to distinguish any external factor which alone could be responsible for the oscillations. Whatever the factor, or factors, involved, the oscillations contribute to the problem of reproducing experiments on thermal resistance.

## Resistance and Acclimation

As all the fish used in the experiment were captured in one pond, the temperature and salinity to which they were acclimated in nature were similar for all the fish. In the laboratory the acclimation to 30 C was common to all fish. Four salinities were used and the regressions computed show the effects of these salinities on the acclimation rates.

At 40.8 C fish at 10 and 20 $^{0}/00$ salinity not only gained acclimation to 30 C more quickly than did fish at 1 and 30 $^{0}/00$ (indicated by visual inspection and the deletion, for lack of significance, of the quadratic term at 10 and 20 $^{0}/00$ salinity) but also had a higher level of resistance to heat (Table 4). The mean asymptotic values (Table 6) were computed from the regression lines and were therefore influenced by the variability in the data. However, except in the case of data at 30 $^{0}/00$ salinity where no asymptote was reached, they gave a good comparative value for looking at the differences between salinities. The combined analysis of variance between salinities for fish killed at 40.8 C (Table 5) show that at at least one salinity the coefficients for the regression equation were significantly different ( $\alpha$ = 0.01). Differential stress among salinities, combined with a common reaction to higher temperatures, could have been the reason for this difference in coefficients.

At 41.4 C no significant difference in coefficients was found from a combined analysis of variance (Table 8). Similar to 40.8, fish killed at 1 and 30 $^{0}/00$ at 41.4 C had the lowest mean asymptotic values. The differential salinity stress found at 40.8 C was apparently masked by the greater stress to temperature created at 41.4 C. The deletion, because of lack of significance, of the quadratic term from the regression equations at 41.4 C only at 10 $^{0}/00$ salinity (Table 7), also strengthens the possibility that "quickly lethal" temperatures might be a masking factor.

## Physiological Variation

One of the problems encountered was the high variability in the survival times of individuals within a sample. This variability has been described as possibly being due to different lethal effects (Neill, Strawn and Dunn, 1966; Allen and Strawn, 1967). The presence of more than one lethal effect implies differences in the physiological makeup of fish within a population. In this study a frequency plot of the regression residuals (Figure 8) show that there is a well defined group of fish that died more quickly than did the majority of fish.

Reduction in variability would be obtained by treating data of this type as two seperate groups of fish. Information as to possible physiological differences within a population might well be used in the culture of this species. Further research is needed to ascertain the number of lethal factors involved.

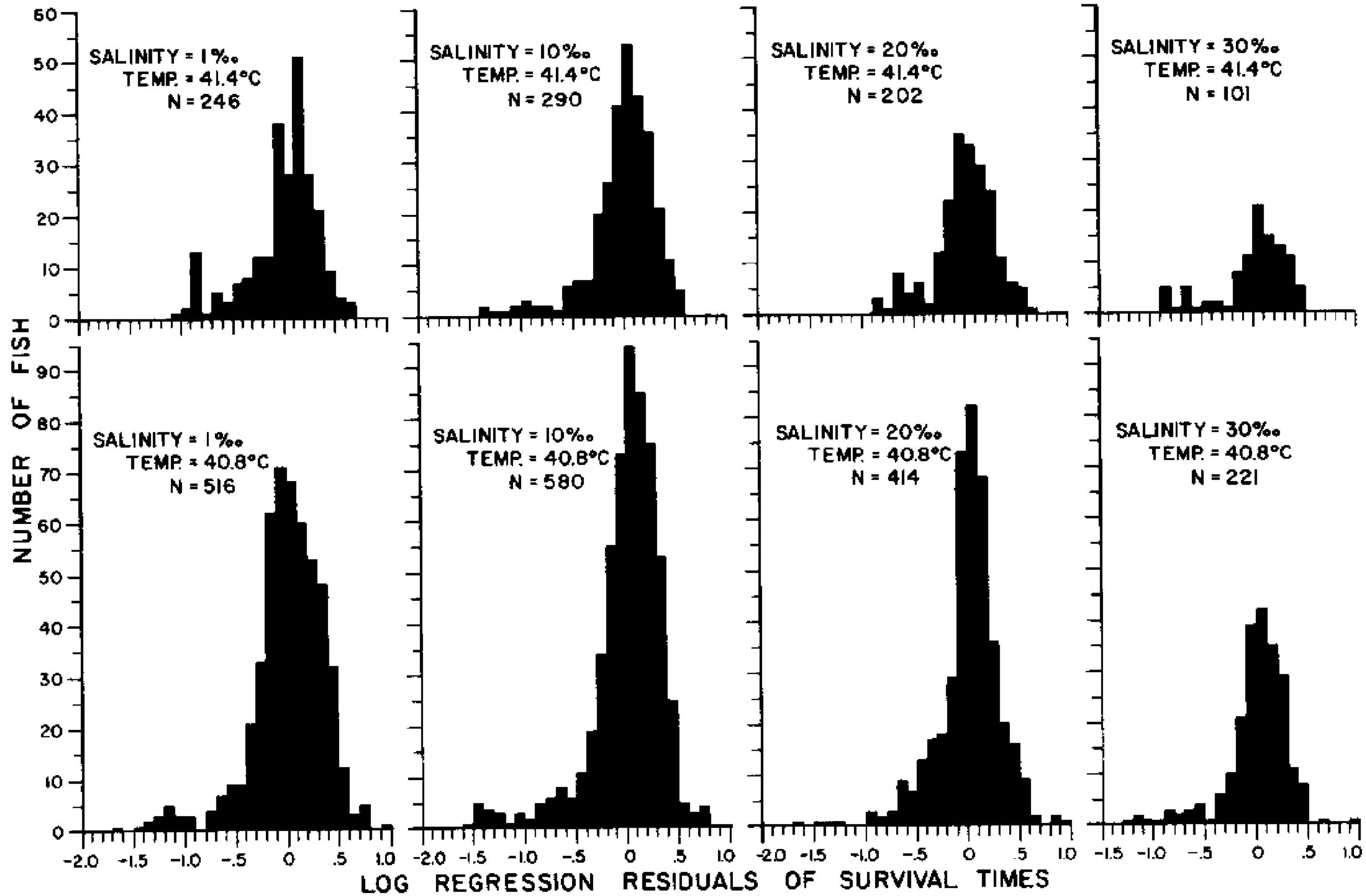


Figure 8. A frequency plot of residuals from the computed regression lines for each temperature-salinity combination. Plot shows lack of normality due in each case, to a group of fish having "short" survival times. Intervals are 0.1 on the log scale. N is equal to the total number of fish killed.

## Additional Factors

The sex and length of the fish were contributing factors only at 1 $^{0}/_{00}$ salinity (Table 9). In this case long males seem to have had the advantage over the others. The short survival times for fish at both kill temperatures at 1 $^{0}/_{00}$ salinity indicated stress which accentuated survival-time differences in sex and length. The positions at the time of death of fish having long survival times in the kill tanks (floating or on the bottom) was different for each of the two kill temperatures (Table 10). This difference could be a result of the causes of death being different at the different lethal temperatures.

## CONCLUSIONS

1. Temperature acclimation took place at a faster rate at 10 and 20 $^{0}/00$ then at 1 and 30 $^{0}/00$ salinity. The difference in rates was the greatest during the first week of acclimation.
2. Complete acclimation was not reached within the test periods except possibly in the case of fish at 10 $^{0}/00$ salinity.
3. Fish at 10 and 20 $^{0}/00$ had a higher level of resistance to heat death then did fish at 1 and 30 $^{0}/00$ salinity.
4. A change in salinity is an important factor in the survival rate when the environmental temperature is raised.
5. Oscillations in the daily mean-survival times are probably due to a complex of factors.
6. Bimodality in the survival times could be due to the presence of more than one physiological group within the population.
7. The sex and length of the fish becomes a factor in survival time under the stress of 1 $^{0}/00$ salinity.

## LITERATURE CITED


Allanson, B. R., and R. B. Noble. 1964. The tolerance of Tilapia mossambica (Peters) to high temperature. Trans. Amer. Fish Soc., 93: 323-332.

Allen, K. O. and K. Strawn. 1967. Heat tolerance of channel catfish, Ictalurus punctatus. Proc. Ann. Conf. S. E. Assoc. Game & Fish Comm., 21: 399-411.

Brett, J. R. 1944. Some lethal temperature relations of Algonquin Park fishes. Univ. Toronto Biol. Ser. 52 (Publ. Ont. Fish. Res. Lab. 64), 5-49.

__________. 1946. Rate of gain to heat tolerance in goldfish. Univ. Toronto Biol. Ser. 54 (Publ. Ont. Fish Res. Lab. 64), 9-28.

__________. 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Biol. 31(2): 76-87.

Brown, M. E. 1957. The Physiology of Fishes. Acad. Press Inc., New York, N.Y. Vol 2: 422.

Doudoroff, P. 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with Girella nigricans (Ayres). Biol. Bull. 83: 219-244.

Hildebrand, S. F. 1919. Notes on the life history of the minnows, Gambusia affinis and Cyprinodon variegatus. Rept. U.S. Comm. Fish., 1917: 1-14.

Hubbs, C. L. 1936. Fishes of the Yucatan Peniusula. Carn. Inst. Wash., Publ. 457: 157-287.

Kilby, J. D. 1955. The fishes of two Gulf coastal marsh areas of Florida. Tulane Stud. Zool. 2(8): 175-247.

Kinne, O. 1960. Growth, food intake, and food cover- sion in a euryplastic fish exposed to different temperatures and salinities. Physiol. Zool. 33(4): 288-317.

_________ and E. M. Kinne. 1962. Rates of development in embryos of a cyprinodont fish exposed to different temperature-salinity-oxygen combinations. Canad. Jour. Zool. 40: 231-253.

Neill, W. H., K. Strawn, and J. E. Dunn. 1966. Heat resistance experiments with the longear sunfish, Lepomis megalotis (Rafinesque). Ark. Acad. Sic. Proc. 20: 39-49.

Raney, E. C., R. H. Backus, R. W. Crawford, and C. R. Robbins. 1953. Reproductive behavior in Cyprinodon variegatus (Lacepede) in Florida. Zoologica, 38(2): 97-104.

Simpson, D. G. and G. Gunter. 1956. Notes on habitats, systematic characters, and life histories of Texas salt water cyprinodontes. Tulane Stud. Zool. 4(4): 113-134.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Lim. & Ocn. 14(5): 799-801.

Strawn, K. and J. E. Dunn. 1967. Resistance of Texas salt- and freshwater-marsh fishes to heat death at various salinities. Tex. J. Sci. 19(1): 57-76.

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15. Supplementary Notes

Dr. Kirk Strawn directed the technical report.

16. Abstracts

The stocking of waters with marine fish for culture frequently involves taking fish from one salinity and temperature and putting them into another. The ability of fish to withstand these changes depends on the rate their metabolic processes can adjust to new levels. *Cyprinodon variegatus* is one of the most resistant of fishes to high temperature and is used in this study. The purpose of this study was to determine the acclimation rates for *cyprinodon variegatus* to 30 C at various salinities. Salinity was used as the variable.

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