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Russell A. Dean

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HIGH TEMPERATURE TOLERANCES OF ANURAN AMPHIBIANS

by

Russell A. Dean

B.A., St. Olaf College 1963

A Thesis

Submitted to the Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota

June
1966

This thesis submitted by Russell A. Dean in partial fulfillment of the requirements for the Degree of Master of Science in the University of North Dakota is hereby approved by the Committee under whom the work has been done.

William D. Schmid
Chairman

Syed M Jalal

Robert W Seabloom

Christopher J. Hamre
Dean of the Graduate School

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I wish to express my sincere appreciation to my advisor, Dr. W.D. Schmid, for his encouragement and help throughout this study.

Research for this thesis was sponsored in part by the Summer Biological Research Program at the Lake Itasca Forestry and Biological Station under terms of National Science Foundation grant #GB3390.

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HIGH TEMPERATURE TOLERANCES OF ANURAN AMPHIBIANS

Russell A. Dean, Master of Science

The thesis here abstracted was written under the direction of William D. Schmid and approved by Syed M. Jalal and Robert W. Seabloom as members of the examining committee, of which Mr. Schmid was chairman.

ABSTRACT

The purpose of this study was to obtain accurate quantitative estimates of the high temperature tolerance of five species of anuran amphibians, *Bufo hemiophrys*, *Rana pipiens*, *R. sylvatica*, *R. septentrionalis*, and *Pseudacris nigrita*. *B. hemiophrys* and *P. nigrita* were collected in the vicinity of Grand Forks, North Dakota; the remaining species were collected at Itasca State Park, Minnesota. Tolerance was measured using the water submersion method described by Schmid (Ecology, 1965). The data were analysed by several statistical methods and the results were compared. The order of tolerance (highest to lowest) was *B. hemiophrys*, *R. pipiens*, *R. septentrionalis*, *P. nigrita*, and *R. sylvatica*. The interspecific tolerance variation of these anurans was correlated with their known ecologies and geographic distributions. There were no intraspecies correlations among variations of tolerance, sex or weight.

Research for this thesis was sponsored in part by the Summer Biological Research Program at the Lake Itasca Forestry and Biological Station under terms of National Science Foundation grant #GB3390.

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William D. Schmid

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INTRODUCTION

Temperature relations are of paramount importance to the complete ecological understanding of any species, and especially of ectotherms. "Cold-blooded" animals can be active only within ranges of environmentally-induced body temperatures to which they are specifically adapted. It may be assumed that thermal factors affect the distribution and the range of these animals. A study by Schmid (1965a) showed a correlation between high temperature tolerance and geographical distribution for two species of amphibians in North Dakota. The purpose of this thesis was to describe accurate quantitative estimates of the high temperature tolerance of five species of anuran amphibians common to northwestern Minnesota. Certain other factors of the ecology of these species have already been studied. Habitat preferences of these species were studied by Marshall and Buell (1955), who found definite relationships between the occurrence of amphibians and the different vegetation zones of a bog succession in Itasca Park, Minnesota. Various aspects of the water economies of these species were studied in the same region by Schmid (1965b).

Five commonly occurring species with widely varying habitat requirements were used in this study. The following descriptions of their preferred habitats were drawn from field observations and from the published work of others (Wright and Wright, 1949; Oliver, 1955; Marshall and Buell, 1955; Stebbins, 1954; and Breckenridge, 1944). Information on the geographical distributions presented here and in Figures 1, 2, 3, 4, and 5 were taken from Breckenridge (1944), Wright and Wright (1949), and Stebbins (1954).

Bufo hemiophrys Cope, the Dakota toad, is a terrestrial-fossorial species restricted to the northern prairies. Although Itasca is outside the range of this species, it was collected and tested in order to tie the results of this study in with a previous temperature tolerance study by Schmid (1965a). Specimens were collected in Polk County, Minnesota about one hundred miles northwest of Lake Itasca.

Pseudacris nigrita Leconte, the swamp tree frog, is a very small semiaquatic frog with an extensive geographical distribution. Although Itasca Park is well within the range of this species, it was not commonly encountered there and most of the specimens were collected in Grand Forks County, North Dakota. Specimens were collected from grassy fields adjacent to low marshy areas and in flooded grassy ditches.

Rana pipiens Schreber, the leopard frog, is semiaquatic and has successfully invaded a number of habitats. Specimens were collected from a wide variety of situations including grassy fields and ditches, banks of streams and lakes, in and around small ponds, and in bogs. Specimens were collected from several areas in Itasca Park, Clearwater

County,Minnesota, and from Grand Forks County, North Dakota.

Rana septentrionalis Baird, the mink frog, is the most aquatic of the species studied. It was found in permanent bodies of water usually just off shore. It was very seldom found out of the water and never far from water. Most of the specimens were collected from Lake Itasca just offshore from floating sedge mats. A few were collected from Mary Lake in Itasca Park.

Rana sylvatica Leconte, the wood frog, is semiaquatic but seems more terrestrial than the leopard frog for it was often found in deciduous woods far from any body of water. Specimens were collected in Tamarack, black spruce, and fir-ash communities and along woodland streams and pond margins in Itasca Park. This frog has the most northerly distribution of the species studied. Its range extends north into Alaska.

Because of the nature of this study, several methods of statistical analysis were applied to the data. The statistics thus derived should supplement the ecological understanding of these species.

METHODS

COLLECTION PROCEDURE

All animals used in this study were collected from their natural habitats described above. When possible, specimens of each species were collected from any and all varieties of habitat situations where they were known to occur. This was done in order to reduce any possible bias due to differential acclimatization in the various habitats occupied by a given species. Collection sites were in Itasca State Park, Minnesota, and in the vicinity of Grand Forks, North Dakota. Sampling was conducted without regard to sex or size. Animals were collected throughout the summer months of 1965, and were tested within two days of capture.

Genus and species, location of capture, and date of capture were recorded for each animal at the time of capture. Total weight, urostyle-snout length, and date were recorded at the time of testing. Observations recorded during the actual tolerance test are discussed below. Immediately upon termination of the tolerance test the deep rectal temperature of the specimen was measured with a YSI* thermistor

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* Yellow Springs Instrument Company Inc.
Address: PO box 279 Yellow Springs, Ohio 45387

thermometer. The extent of recovery was noted and the sex of the specimen was determined and recorded before the specimen was discarded.

TEST PROCEDURE

Animals were randomly allocated to test temperature groups. To determine the high temperature tolerance each animal was submersed in a warm water bath of a specific temperature which was maintained until the end of the test. Submersion in warm water eliminated the cooling effect of evaporative water loss from external skin surface which permitted closer regulation of the test temperature.

The actual test chamber was a 600 ml. beaker full of water. The beaker was set in a warm water bath of a certain test temperature. The temperature within the beaker was allowed to equilibrate with the temperature of the surrounding bath before the frog was introduced. A glass plate covered the bath so that only the snout of the animal could remain above water. A stopwatch was used to time the responses of the animal in the bath.

The survival time was recorded as the length of time from introduction to expiration of the individual as defined below. The time of other events and behavior pattern such as swimming activity, muscle spasms, breathing movements, and heartbeat were also recorded. If an animal survived exposure of three hours the test was terminated since some of these species come under hydration stress if left submersed for long periods of time (Schmid, 1965b).

CHOICE OF END POINT INDICATOR

To define the tolerance characteristics of each species relative to the other species tolerances, the same testing procedure was used for all animals; but to obtain meaningful data it was also imperative that a valid end point be used. Ideally the end point for lethal temperature tolerance would be the instant of expiration. The actual instant of death in these amphibians was obscure so an indicator was sought which was consistently close to the actual death point. Several end point indicators described in previous studies of temperature tolerance could have been used on one or more of these species. However, it was evident after a few preliminary tests that behavior patterns near death varied qualitatively from species to species and also from temperature to temperature.

The only single indicator observed to be valid in every test was the point at which the animal lost irritability to a mechanical stimulus. This was a quantal response which was close to the death point at all test temperature levels and for all five species. The stimulus used was a pinch of the animal's toes with a forceps. If the animal responded it was left in the bath. The end point recorded was the earliest time at which a stimulus would not provoke a response. When working with a particular species the investigator could tell approximately when this time was approaching by the pattern of behavior leading up to the end point.

The indicators most commonly used in previous work were not valid for this work. Cowles and Bogert (1944) described the "critical thermal maximum" as the "thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to death". The onset of muscle spasms or the loss of righting ability has been commonly used as an end point to indicate the "critical thermal maximum" (CTM). These and other possible end points are listed below with brief evaluations of their validity as indicators of the actual time of expiration.

1. Cessation of body movements.--This point was easily observed but had a highly variable relationship to the death point.

2. Loss of righting ability.--This was impossible to use with the water bath method without disturbing the test situation.

3. Cessation of breathing.-- This point was quite difficult to observe because breathing was often discontinuous.

4. Muscle spasms.-- This response usually did not occur at the lower test temperatures.

5. Convulsions.--This response varied greatly with species and test temperature.

6. Rigidization.--Rigidization occurred at or before death at the higher test temperatures; at lower temperatures it occurred after death or not at all.

7. Cessation of heartbeat.--This point was hard to observe; the

rate and continuity of heartbeat changed considerably prior to complete cessation.

8. Parotoid gland secretion.--This response was good for toads, but frogs have no similar structures.

9. Loss of irritability.--This was a quantal response close to the death point throughout the range of high lethal temperatures and equally valid for all five species.

RESULTS - STATISTICAL ANALYSIS OF DATA

Survival times were recorded in minutes. Most of the statistical tests were applied to the logarithm of the original data. This logarithmic transformation was used because the logarithm of the survival time was more normally disributed $\left[\log \text{time} \overset{d}{=} N(\mu, \sigma^2)\right]$ than were the raw data on an arithmetic scale. Many statistical methods are based on the assumption that the population of measurements is distributed approximately normally. The survival time of each animal is shown in Tables 1, 2, 3, 4, and 5 along with the transformed values.

TOLERANCE CURVES

The high lethal temperature tolerances for a range of temperatures are presented for each species in Figures 6-10. The survival time of each individual is plotted logarithmically against the test temperature.

Figures 11-15 are statistical representations of the logarithms of the values shown in Figures 6-10. The extended lines represent the range of observed values at each test temperature. The open bars represent the 95 per cent confidence interval of the mean $(\pm t_{.975}^{df})(SE_{\bar{x}})$. The filled circles represent the geometric mean tolerance value at each temperature.

FIG. 6

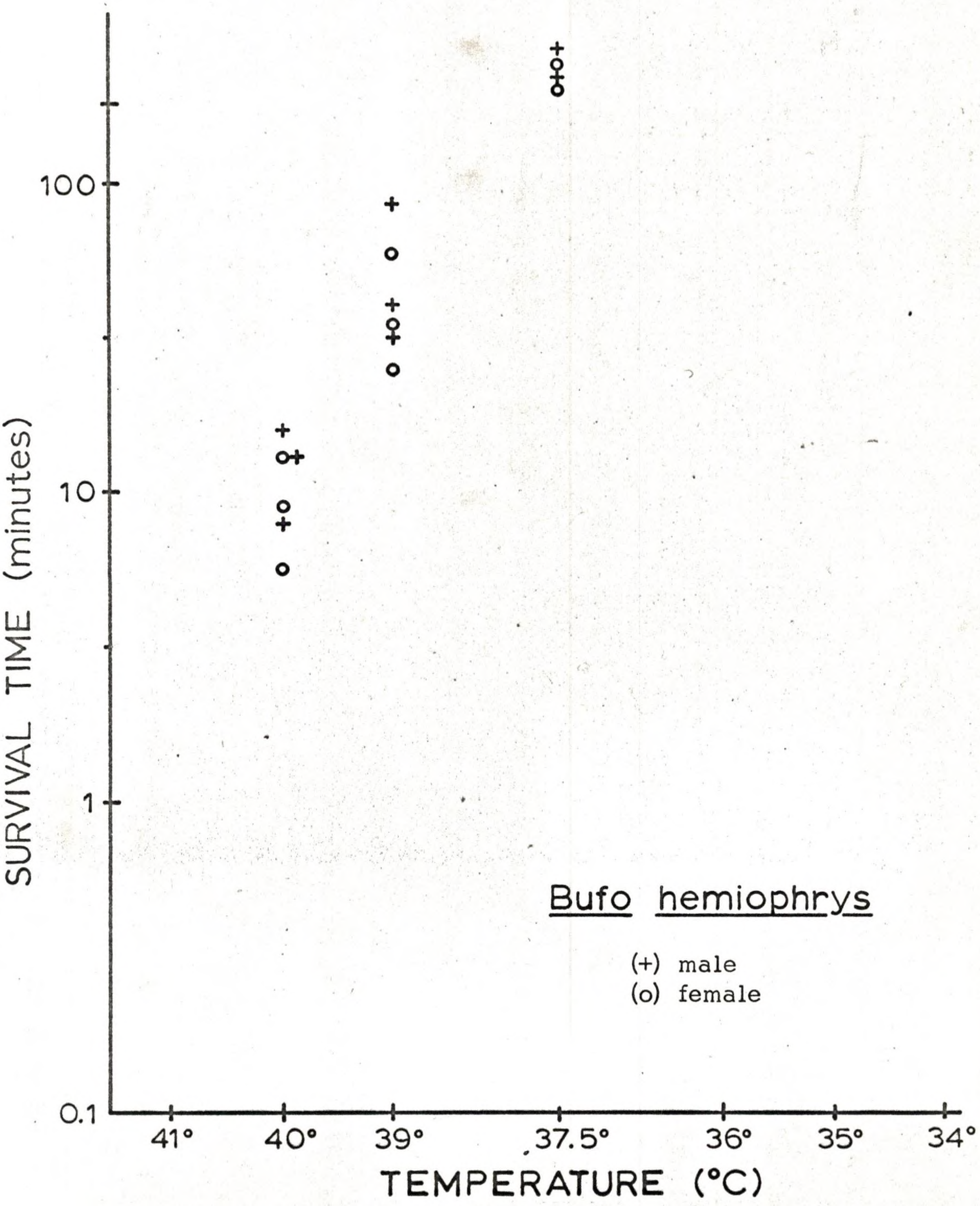

SURVIVAL TIME (minutes)
100
10
1
0.1
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)
Bufo hemiophrys
(+) male
(o) female

FIG. 7

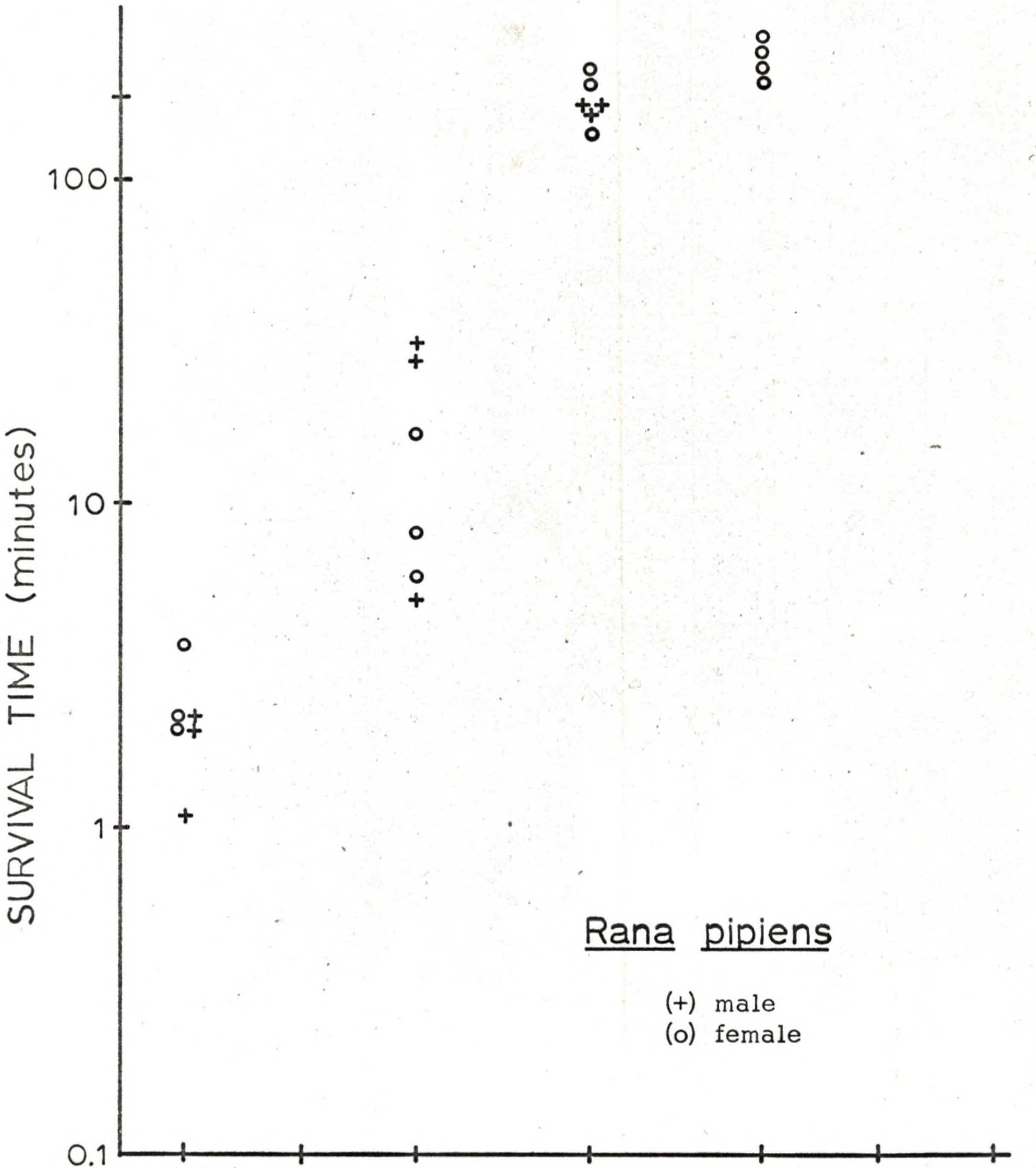

SURVIVAL TIME (minutes)
100
10
1
0.1
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)
Rana pipiens
(+) male
(o) female

FIG. 8

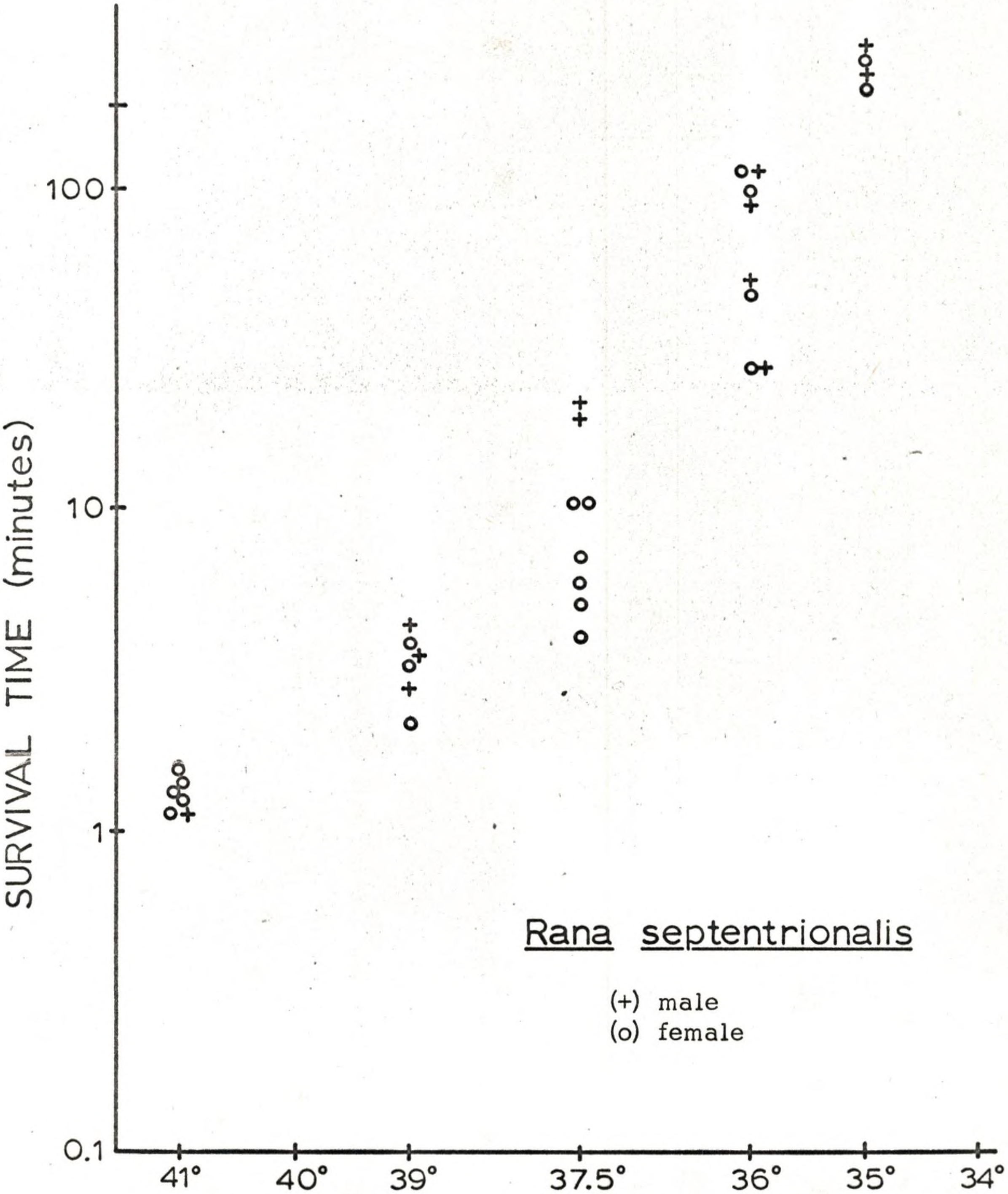

SURVIVAL TIME (minutes)
100
10
1
0.1
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)
Rana septentrionalis
(+) male
(o) female

FIG. 9

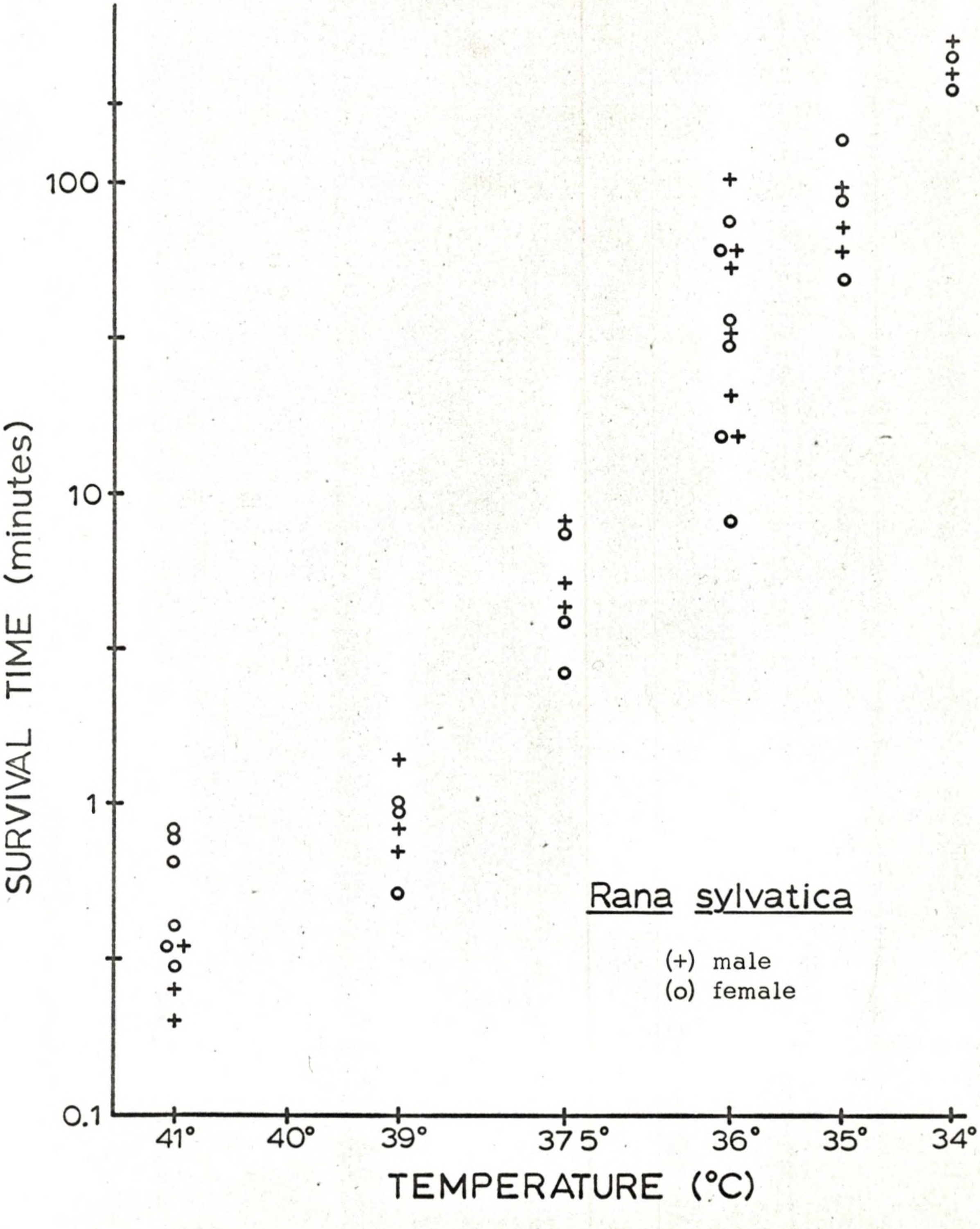

SURVIVAL TIME (minutes)
0.1
1
10
100
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)
Rana sylvatica
(+) male
(o) female

FIG. 10

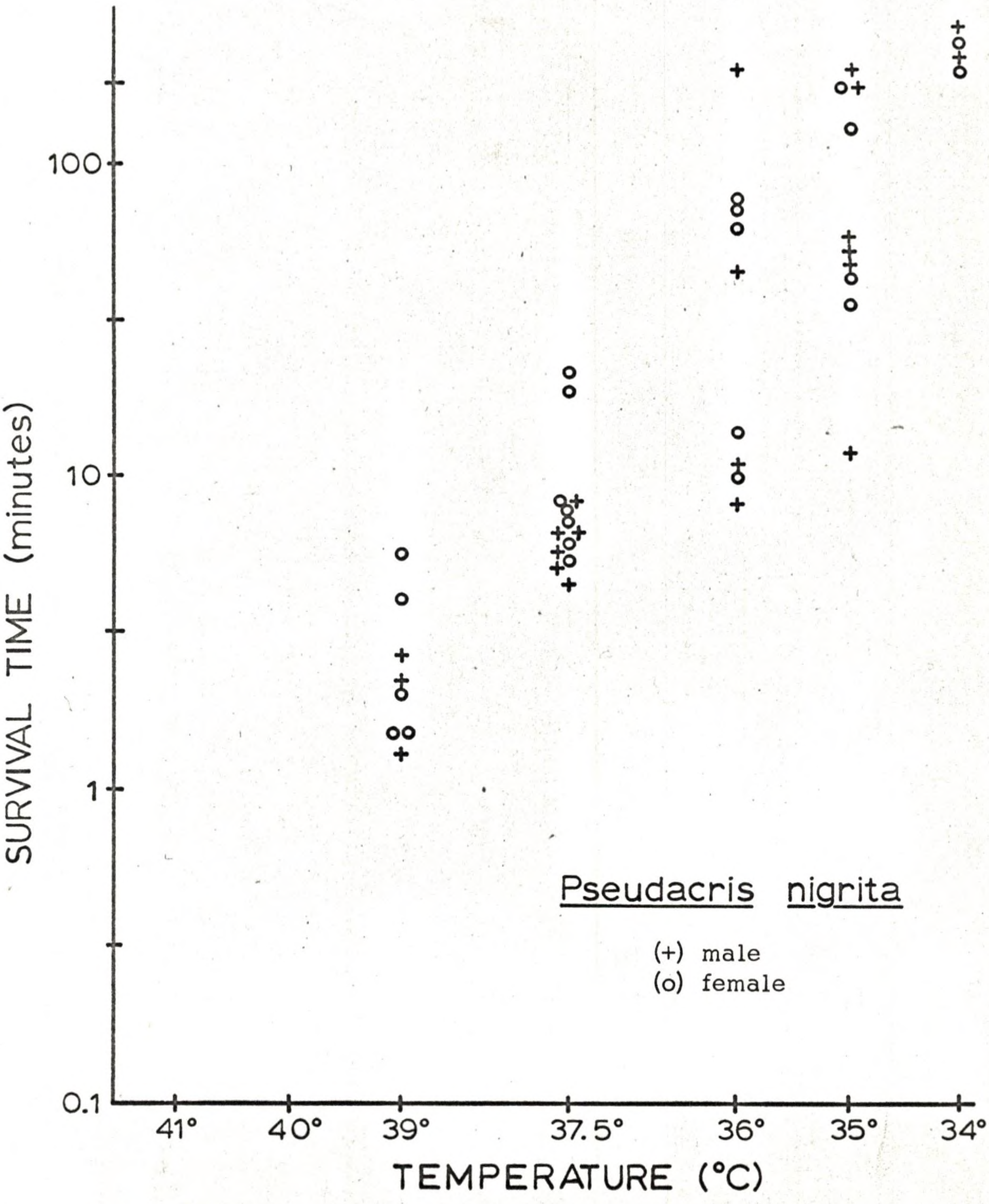

SURVIVAL TIME (minutes)
100
10
1
0.1
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)
Pseudacris nigrita
(+) male
(o) female

FIG. 11

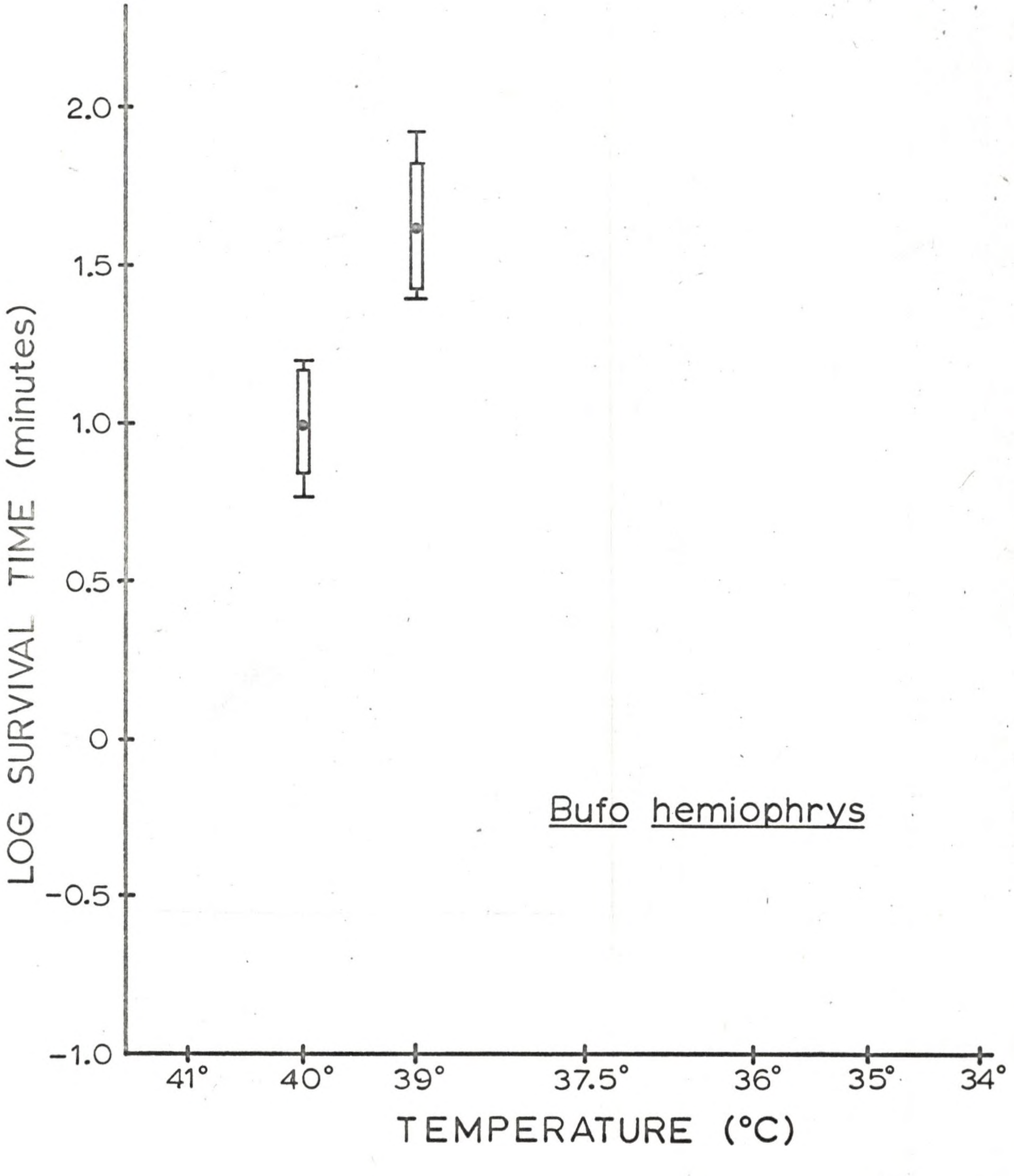

LOG SURVIVAL TIME (minutes)
2.0
1.5
1.0
0.5
0
-0.5
-1.0
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)
Bufo hemiophrys

FIG. 12

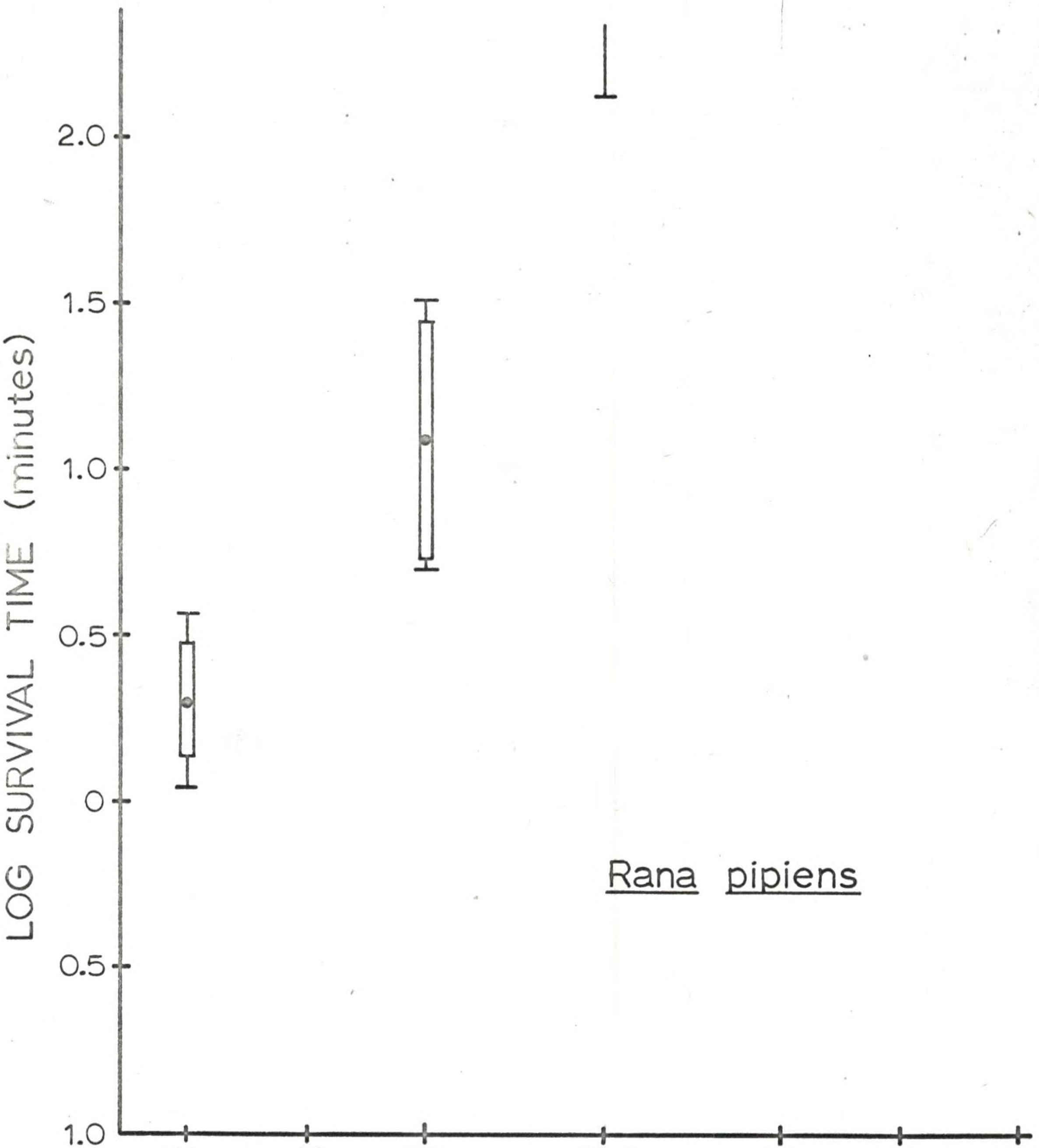

LOG SURVIVAL TIME (minutes)
2.0
1.5
1.0
0.5
0
0.5
1.0
41
40
39
37.5
36
35
34
TEMPERATURE (°C)
Rana pipiens

FIG. 13

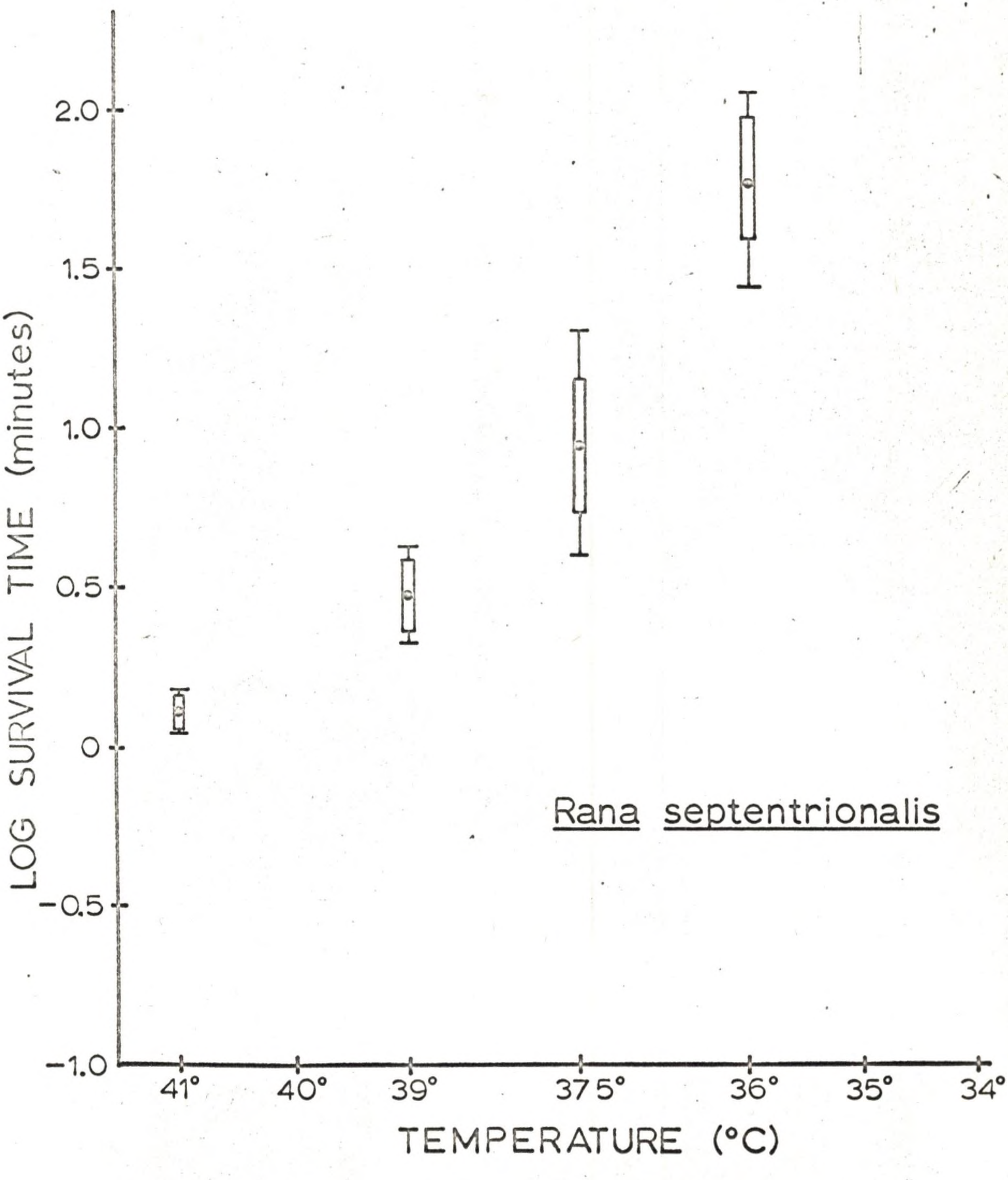

2.0
1.5
1.0
0.5
0
-0.5
-1.0
LOG SURVIVAL TIME (minutes)
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)
Rana septentrionalis

FIG. 14

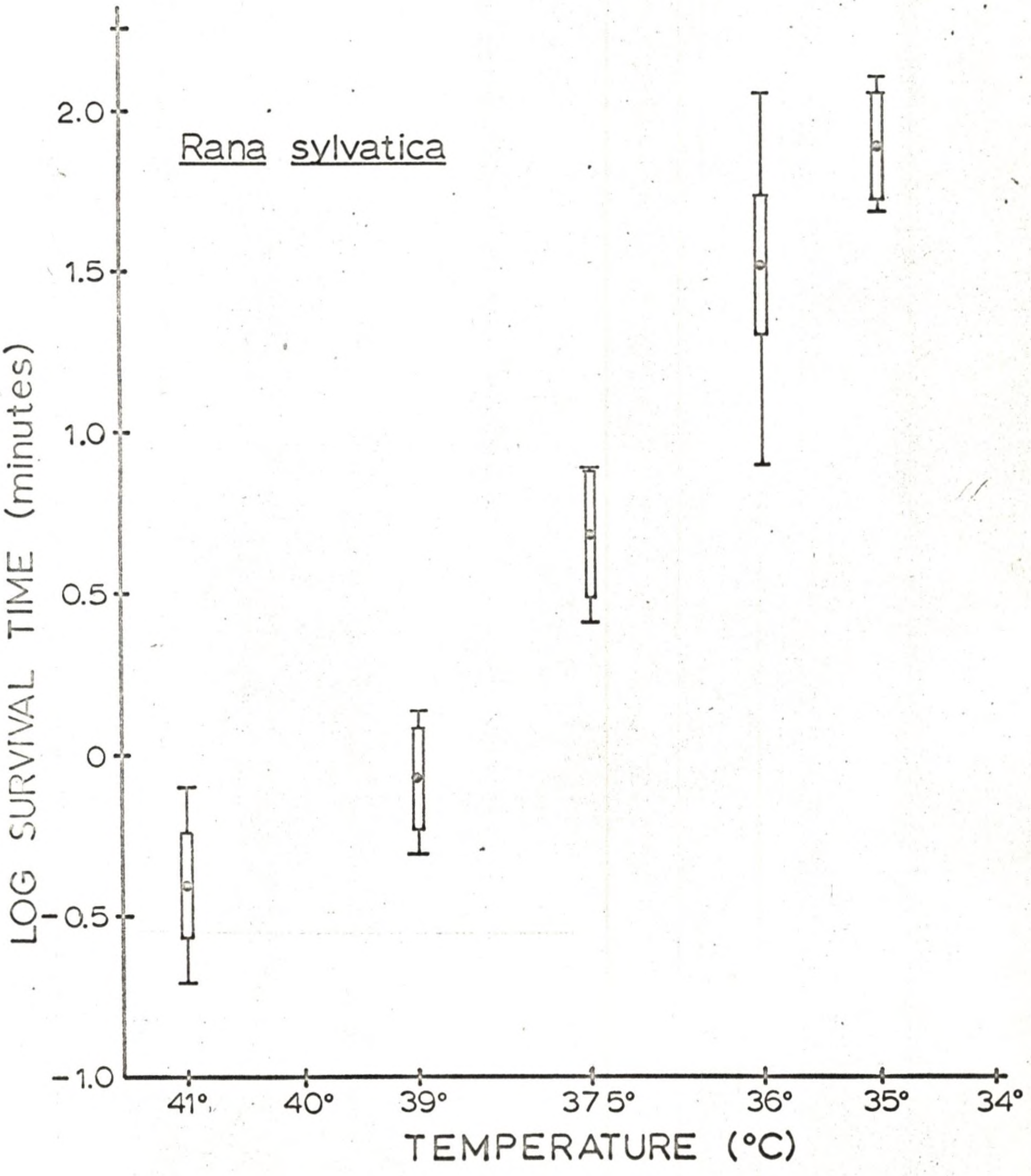
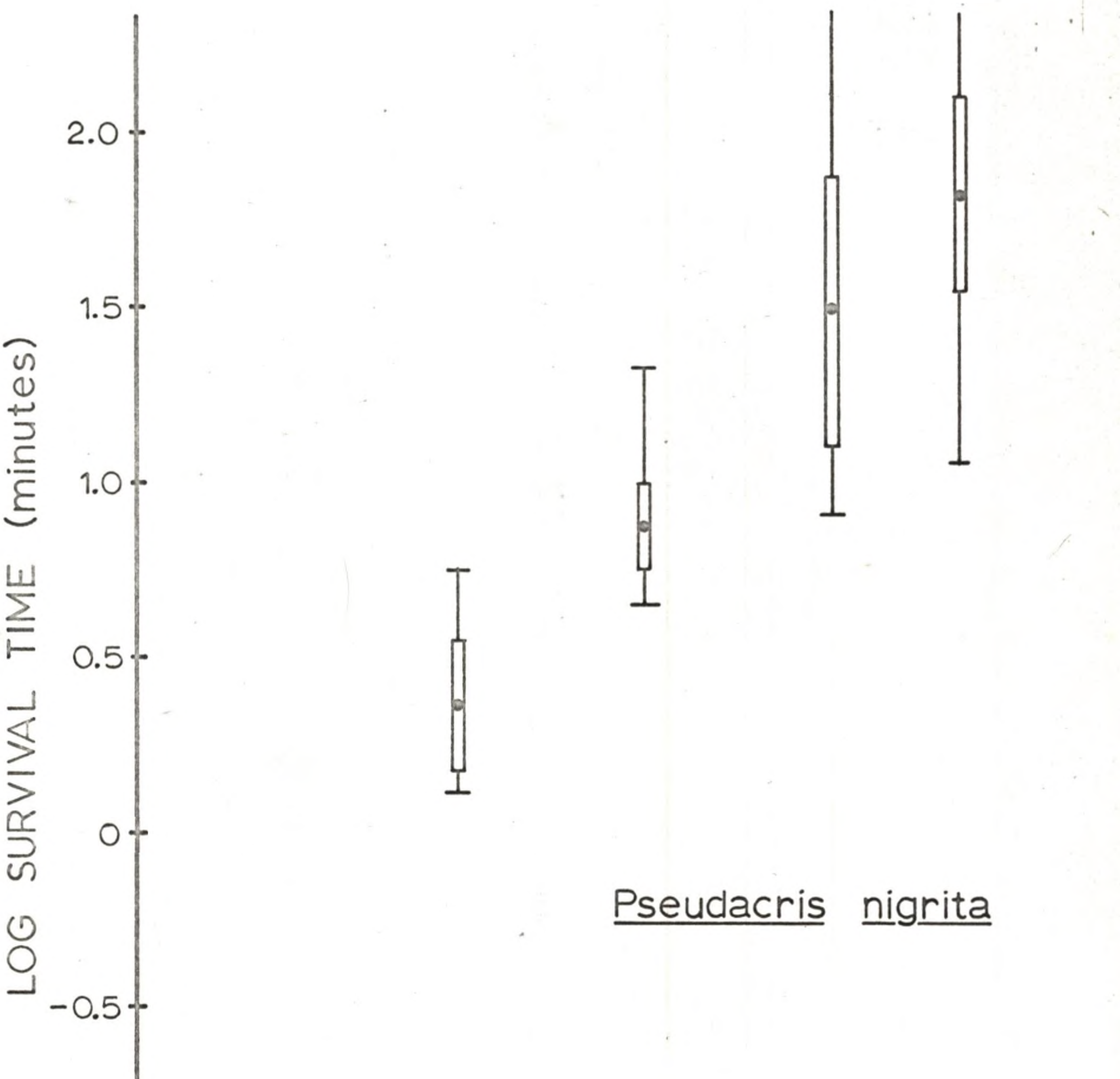

Rana sylvatica
LOG SURVIVAL TIME (minutes)
2.0
1.5
1.0
0.5
0
-0.5
-1.0
41°
40°
39°
37.5°
36°
35°
34°
TEMPERATURE (°C)

FIG. 15


LOG SURVIVAL TIME (minutes)
2.0
1.5
1.0
0.5
0
-0.5
-1.0
41°
40°
39°
37 5°
36°
35°
34°
TEMPERATURE (°C)
Pseudacris nigrita

INTERSPECIFIC COMPARISON OF TOLERANCES

Lethal temperature tolerances of the differentspecies are compared by various methods in Figures 16 and 17 and in Tables 6, 7, and 8. In Figure 16 the mean tolerances of all five species are plotted for comparison. The order of tolerance to high temperature appears to be Bufo hemiophrys (most tolerant), R. pipiens, R. septentrionalis, P. nigrita, R. sylvatica (least tolerant). The significance of the distances between these curves is not apparent in this figure.

The 39°C test level was the lowest temperature at which specimens of all five species expired within the three hour limit. Figure 17 was extracted from Figures 11-15 and represents the 39°C statistics for each species. The open bars represent the 95 per cent confidence interval for each mean so non-overlapping bars represent statistical differences between the means at the 5 per cent level of significance. At this temperature level the three species of the genus Rana were significantly different from each other. The intervals of B. hemiophrys and R. pipiens overlap, and P. nigrita overlaps R. septentrionalis.

Another simple comparison of the tolerances of these species is illustrated in Table 6 where the percentage of individuals surviving beyond the three hour test limit is shown for each test group. A similar comparison of percentage survival after one hour of exposure is presented in Table 7. By this method of comparison the three species of the genus Rana had apparently different tolerances, R. pipiens

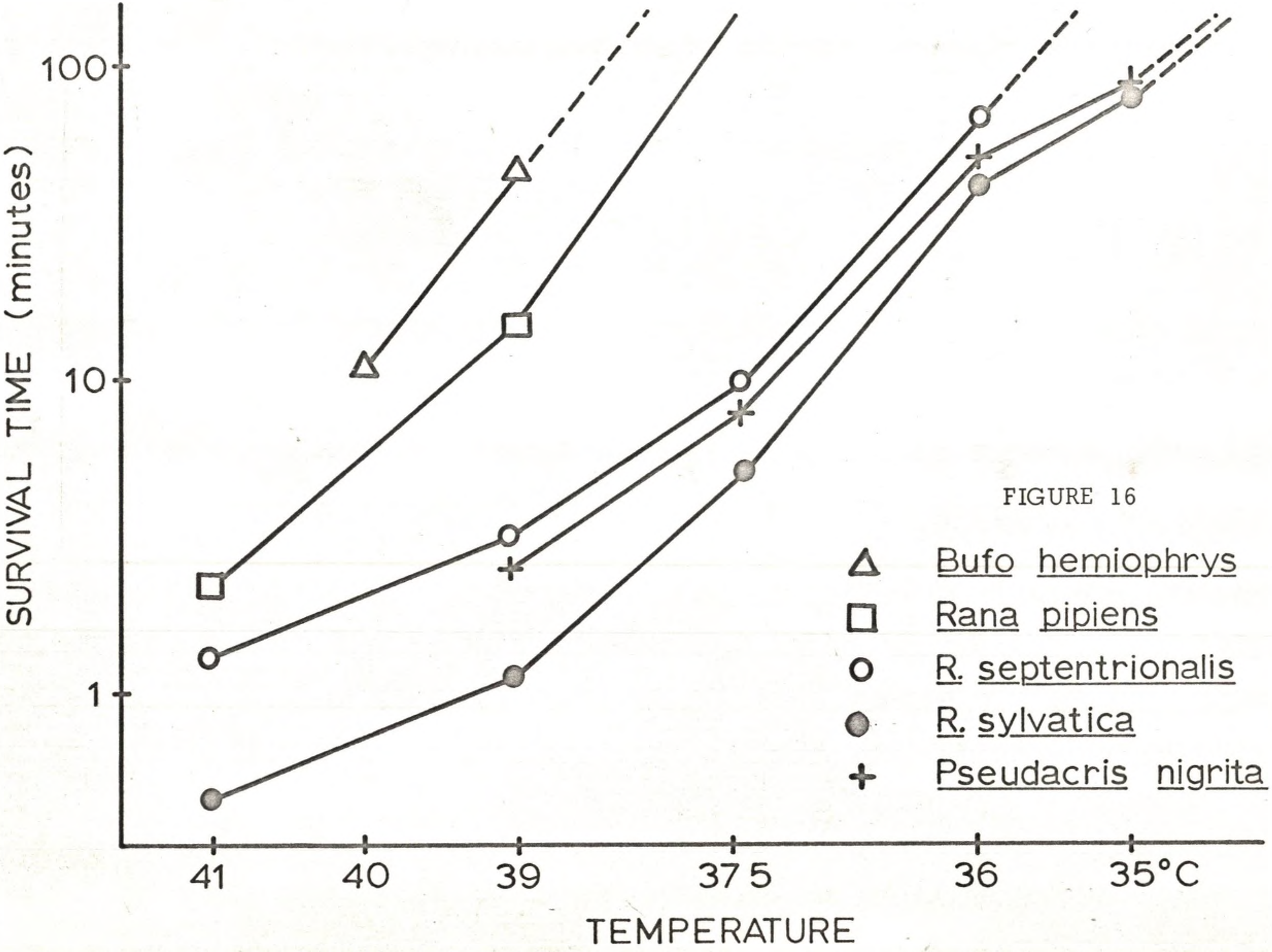

SURVIVAL TIME (minutes)
100
10
1
41
40
39
37.5
36
35°C
TEMPERATURE
△ Bufo hemiophrys
□ Rana pipiens
○ R. septentrionalis
● R. sylvatica
+ Pseudacris nigrita

FIGURE 16

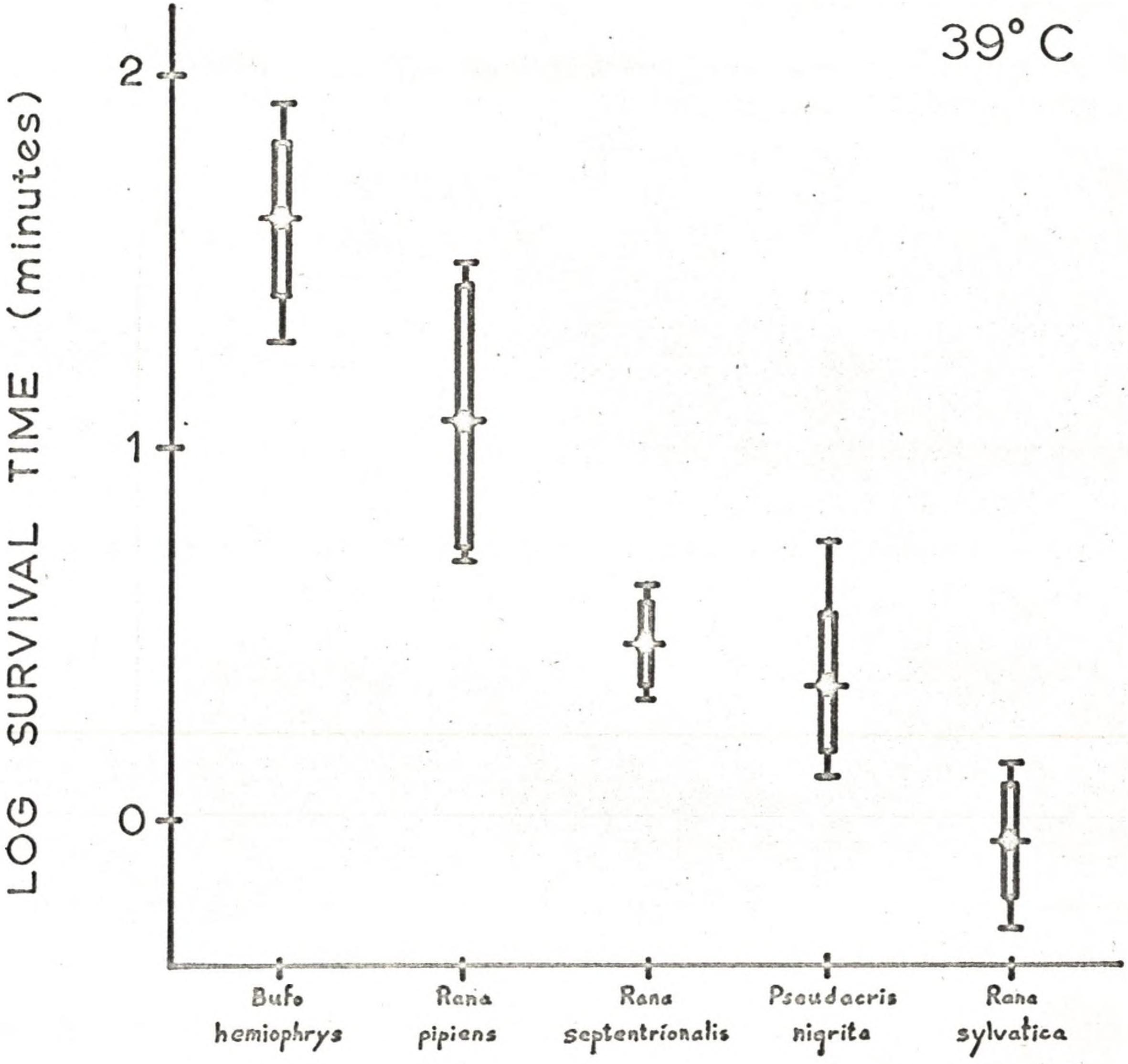


Fig. 17.--95 per cent confidence intervals at 39°C

being the most tolerant and *R. sylvatica* the least tolerant as in Figure 17. Here the order of tolerance appears the same and no clear distinction can be drawn between tolerances of *B. hemiophrys* and *R. pipiens*, However, *R. sylvatica* and *P. nigrita* do not appear as different by this comparison as by the 39°C confidence intervals.

TABLE 6.--Per cent survival after three hours exposure

Species	39°	37.5°	36°	35°	34°C
Bufo hemiophrys	0	100	-	-	-
Rana pipiens	0	33	100	-	-
R. septentrionalis	0	0	0	100	-
R. sylvatica	0	0	0	0	100
Pseudacris nigrita	0	0	10	10	100

TABLE 7.--Per cent survival after one hour exposure

Species	40°	39°	37.5°	36°	35°	34°C
Bufo hemiophrys	0	17	100	-	-	-
Rana pipiens	0	0	100	-	-	-
R. septentrionalis	0	0	0	50	100	100
R. sylvatica	0	0	0	25	75	100
Pseudacris nigrita	0	0	0	45	40	100

There are several non-parametric methods which afford another approach to the calculation of high thermal tolerance estimates. These methods were used to estimate the LD_{50} temperature (the temperature level at which 50 per cent of the animals would expire within one hour) for each species. Results obtained by these methods (Table 8) agreed closely with each other. However, these quantal response estimates

TABLE 8.--LD_{50} temperature estimates obtained by non-parametric methods*

Species	Spearman-Kärber	Reed-Muench	Dragstedt-Behrens	Thompson moving average	Gaddum extreme
Bufo hemiophrys	38.82°C	38.7	38.63	38.66	38.67
Rana pipiens	38.25	38.25	38.25.	38.25	38.25
R. septentrionalis	36.12	36.0	36.0	36.0	36.12
R. sylvatica **	35.56	35.5	35.50	35.5	35.52
Pseudacris nigrita	35.45	35.3	35.35	35.35	35.45

* Each value is an estimate of the temperature level at which exactly 50% of a sample will expire within 60 minutes

** Sample calculations for each estimator of this species are given in the Appendix seem to contradict the quantitative method because *P. nigrita* appears to have a lower tolerance than *R. sylvatica*.

A parallel-line assay technique described by Finney (1952) was used to estimate the horizontal distance (°C) between the tolerance curves of different species. The results (Table 9) show that the upper thermal tolerance of *R. pipiens* was 1.27 to 3.01°C higher (95 per cent confidence interval) than that of *R. septentrionalis*, 1.45 to 3.15°C higher than that of *P. nigrita*, and 1.68 to 3.48°C higher than that of *R. sylvatica*. This type of estimate is a much more useful tool for ecological and genetic studies because large portions of the tolerance curves are used, not just one temperature level or one time interval which may give inaccurate and conflicting

TABLE 9.--High temperature tolerance difference estimates by the parallel-line assey (Finney, 1952)

Species	Horizontal distance between curves	95% confidence interval estimate of distance
R. pipiens and *R. septentrionalis*	2.14 + 0.43°C	1.27 to 3.01°C
R. pipiens and *R. sylvatica*	2.57 + 0.45°C	1.68 to 3.48°C
R. pipiens and *P. nigrita*	2.30 + 0.43°C	1.45 to 3.15°C
R. septentrionalis and *R. sylvatica*	0.43 + 0.47°C	-0.50 to 1.36°C

results. Also, this technique emphasizes tolerance differences between species and is less concerned with the actual tolerance to an extreme temperature.

The bioassay tolerance estimates of this study and of Schmid (1965a) were combined and plotted against the geographical (latitudinal) distribution of the species (Figure 18). The observed high temperature tolerances of these species are correlated with their geographical distributions; the more southerly species of a family being the more tolerant. There appears to be approximately 3.5°C tolerance difference between bufonid species and ranid species representative of the same latitudinal distribution.

CORRELATIONS BETWEEN TOLERANCE AND INTRASPECIFIC VARIABLES

The above results assume that the only variable influencing the tolerance of any animal was the species of that animal. Since it was possible that variability within species groups could have influenced the results, intraspecies variables were checked.

In Figures 6-10 the survival time of each male specimen is represented by a "+" and the survival time of each female is represented by a "o". Observation of these figures revealed no consistently higher or lower tolerance for either sex. This seemed obvious so no more elaborate test was made.

There was considerable variation in size among the individuals

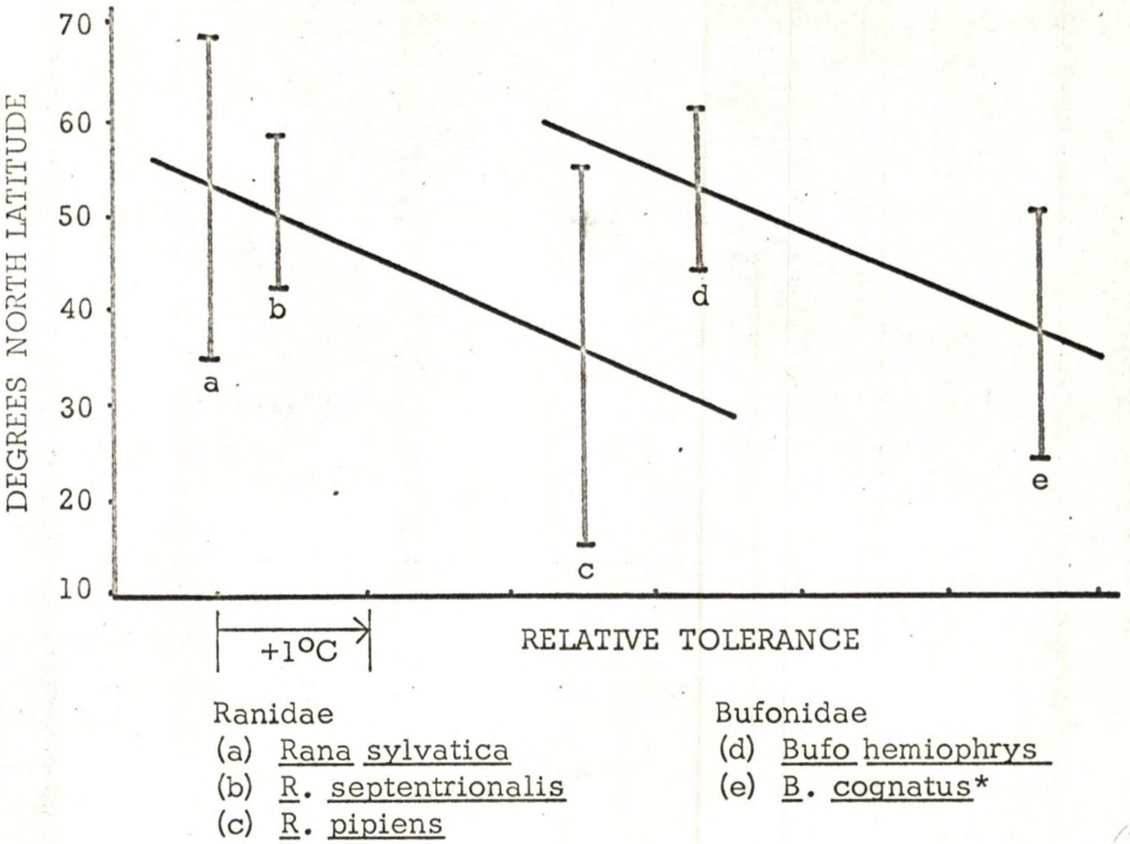


Fig. 18.--Relationship of species high thermal tolerance to species geographical distribution. The vertical lines represent the north-south extension of the range. Diagonal lines connect the midpoints of the vertical lines of each family. Information on the distributions of these species was taken from Breckenridge (1944), Stebbins (1954), and Wright and Wright (1949).

* Tolerance relationship between *B. hemiophrys* and *B. cognatus* was taken from Schmid (1965a).

of some species and between species (Table 10). However, since intraspecific weight differences had no apparent relationship (Table 11) to thermal tolerance, no adjustment of the tolerance curves was made for the weight difference between species.

TABLE 10.--Species weights

Species	Mean (g)	Range
Bufo hemiophrys	2.28	1.1 to 4.8
Rana pipiens	11.2	5.7 to 41.0
R. septentrionalis	12.9	4.6 to 29.8
R. sylvatica	5.6	1.3 to 18.1
Pseudacris nigrita	0.72	0.3 to 1.25

TABLE 11.--Correlation coefficients for weight versus high temperature tolerance

Species		Correlation coefficient r
Bufo hemiophrys		-.142
Rana pipiens		-.294
R. septentrionalis	(35-37.5°C)	.062
	(39-41°C)	.292
R. sylvatica		.067
Pseudacris nigrita		.288

No r values were significantly different from zero at $P \geq .95$

DISCUSSION

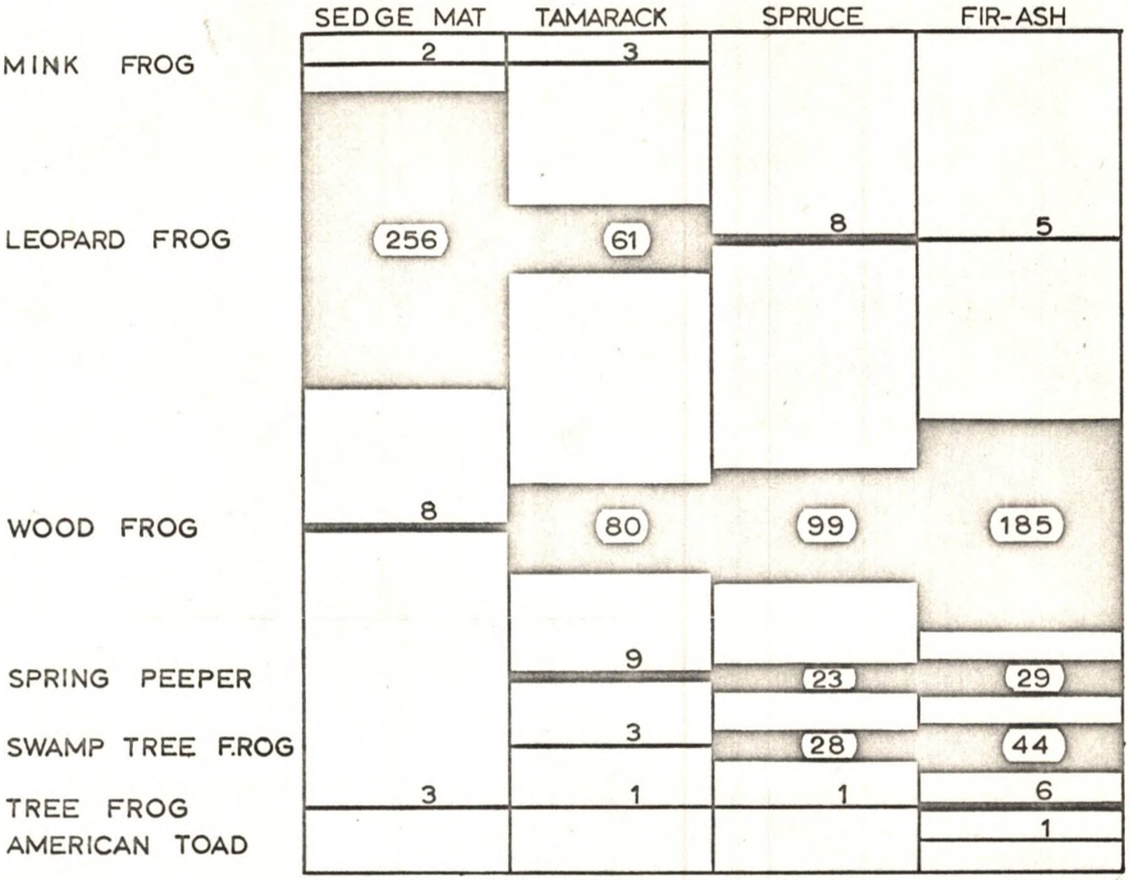
RELATIONSHIP OF OBSERVED TOLERANCES TO SPECIES ECOLOGY

The high temperature tolerance results shown above are consistent with the known ecology and water economies of these species. Figure 19 (from Marshall and Buell, 1955) shows the distribution of frogs found in a bog succession at Itasca Park, Minnesota. Note that the frogs found during that study occurred in habitat situations similar to those described in the introduction to this paper. *R. pipiens* occured in the open grassy area, *R. sylvatica* in the forest, and *R. septentrionalis* in open water. Microclimate data from the same bog are shown in Table 12. These data show certain distinct differences

TABLE 12.--Microclimatic data from three stations of Bog D, Itasca Park, Minnesota; July 30 through August 5, 1953

	Mean Seven-day Temperatures				Vapor Pressure Deficit
	Air		Substratum		6 p.m. August 1
	Max.	Min.	p.m.	a.m.	
Sedge Mat	83	54	73.8	70.2	9.5
Tamarack	76	58	63.6	61.8	7.8
Fir-Ash	72	58	63.0	61.1	4.7

after Marshall and Buell (1955)



(From Marshall and Buell, 1955)

Fig. 19.--Distribution of amphibians in Bog D, Itasca Park, Minnesota. (Mink frogs were often observed in the open water adjacent to the sedge mat).

between the sedge mat and the other zones. The maxima and range of temperature and the vapor pressure deficit are greater in the sedge mat than in the other zones. The substratum temperature is also considerably higher on the sedge mat.

One might guess that species typically occupying the sedge mat would be more tolerant of higher temperatures than would species which prefer the adjacent zones. The results of the present study lend strong support to this hypothesis. *R. pipiens* , the only amphibian found in large numbers in this zone, was significantly more tolerant of high temperatures than the species typically found in the adjacent zones.

To avoid this "hot" zone an animal could select the cooler less variable environment of the moist shaded forest floor (*R. sylvatica* and *P. nigrita*); or it could inhabit the open water offshore from the sedge mat (*R. septentrionalis*) thus utilizing the unique thermal properties of a water environment which would have lower temperature maxima and less temperature variation than the open air environment (Odum, 1959).

Why some species take to the woods while *R. septentrionalis* occupies the open water to avoid extreme temperature variations may be partially explained by the different susceptibility of these species to hydration stress. Schmid (1965b) showed that the more terrestrial species (*R. sylvatica* and *P. nigrita*) had much lower tolerance to hydration stress than the more aquatic *R. septentrionalis*; conversely, *R. septentrionalis* was less tolerant to desiccation than the terrestrial species.

Heatwole, Mercado, and Ortiz (1965) also described a correlation between high temperature tolerance and preferred habitat conditions for two species of frogs in Puerto Rico. *Eleutherodactylus richmondi* inhabits only cool, wet montane forests and had a significantly lower tolerance (CTM) than *E. portoricensis* which inhabits a wide variety of situations from xeric scrub to mossy forests. Their results also agree with this study because size had no effect on the observed CTM for those species.

Besides the correlation of high thermal tolerances with habitat preferences it was shown by this study and by Schmid (1965a) that the thermal tolerances of the anuran species studied are correlated with the latitudinal distribution of the species when compared with other species of the same family. The fact that there is a significant tolerance difference between species of different families representative of the same latitude suggests that the mechanisms involved in high thermal tolerance may have evolved along separate pathways in these two anuran families.

RELATIONSHIP OF OBSERVED TOLERANCES TO SPECIATION AND GENETICS

The actual causes of thermal death in animals are obscure, and no single factor has been delimited. Dill (1938) suggested a derangement of function within the nervous system from hypoxia due to decreased oxygen affinities of the blood. In this regard, Dawson (1960) showed that oxygen demands of the desert iguana at high temperatures appear to be less than those of other lizards of comparable size.

Performance of various tissues in vitro appears to be correlated with the thermal relations of the poikilotherms in nature. Studies by Licht (1964a) demonstrated that intraspecific differences in the thermal relations of muscle are correlated with the extent of thermophily of the intact animal. The incipient lethal temperatures (CTM) of five species of lizards corresponded closely to the lowest temperature at which their muscles were irreversibly damaged. Furthermore, the mean preferred body temperature was closely related to the optimal temperature for in vitro muscle contraction.

Interspecific divergence of thermal tolerances has also been demonstrated in the thermostabilities of sciatic nerves (Adensamer, 1934) and cardiac muscles (Dawson, 1960) from lizards. Experiments by Battle (1926) on skate, _Raja_, tissues and work of Orr (1955) on _Rana pipiens_ tissues suggest that variations in the heat tolerance of species are reflected in the thermostabilities of a wide array of tissue proteins, the myo-neural junctions being the most heat sensitive. Somatic musculature tends to be more heat resistant than the intact animal, in that muscular contractions can be electrically induced in lizards previously paralized by exposure to lethal temperatures (Licht, 1964a). However, even though the muscles may not be the limiting factor for individual heat tolerance, some adjustment in the thermostability of this tissue appears necessary to permit the evident interspecies diversity in tolerance limits.

One of the principal factors determining the behavior of living organisms at high temperatures is the stability of their proteins. Thermal death might be linked with coagulation of proteins or inactivation of enzymes at the high temperature required to produce injury or death. In view of the importance of enzymatic reactions to the temperature dependence of physiological processes, biochemical adjustments with respect to temperature might play an important role in the environmental thermal relations of organisms. The hydrolysis of ATP by ATP-ase is important in muscle contraction. Interspecific variations in the thermostability of ATP-ase seems to underlie the differences found in the thermostability of whole skeletal muscle from various lizard species studied by Ushakov (1958), Licht (1964a), and Ushakov and Darevskii (1960). Licht (1964b) found that the thermal level for the maximum ATP-ase activity in vitro was directly correlated with the level of body temperature preferred for activity by the intact animals of eight different species (lizards). Other enzymes from the same animals do not necessarily share this relationship to organismal thermal tolerances. In contrast to the ATP-ase, there was virtually no difference in the temperature dependence of alkaline phosphatase activity in intestinal homogenates from four species of lizards studied by Licht (1964b).

Licht's results indicate that adjustments at the subcellular level may play an important role in thermal adaptation of poikilotherms, but there are at least two distinct types of enzymes in this respect. One, such as alkaline phosphatase from the intestine, is relatively thermostable and if adaptive modifications have occurred in this enzyme they probably involve the level of specific activity. The second type, exemplified by myosin ATP-ase, appears to have undergone extensive shifts in temperature dependence. The mutability of the latter more thermolabile type of enzyme would probably be a primary requisite for adjustments to increasing temperature unless alternative enzymic pathways can be utilized.

SUMMARY AND CONCLUSION

This thesis has described the high temperature tolerances of five species of anuran amphibians, _Bufo hemiophrys_, _Rana pipiens_, _R. septentrionalis_, _R. sylvatica_, and _Pseudacris nigrita_. Tolerance was measured using a water submersion method. Loss of irritability was found to be the most valid end point indicator for these species and for the temperature levels used. The data were analyzed by several statistical methods and the results were compared. The order of tolerance (highest to lowest) was _B. hemiophrys_, _R. pipiens_, _R. septentrionalis_, _P. nigrita_, and _R. sylvatica_. The interspecific tolerance variation of these anurans was correlated with their known ecologies and geographical distributions. There were no intraspecies correlations between high temperature tolerance variations and sex or between tolerance variations and body weight.

APPENDIX I

DISTRIBUTION MAPS

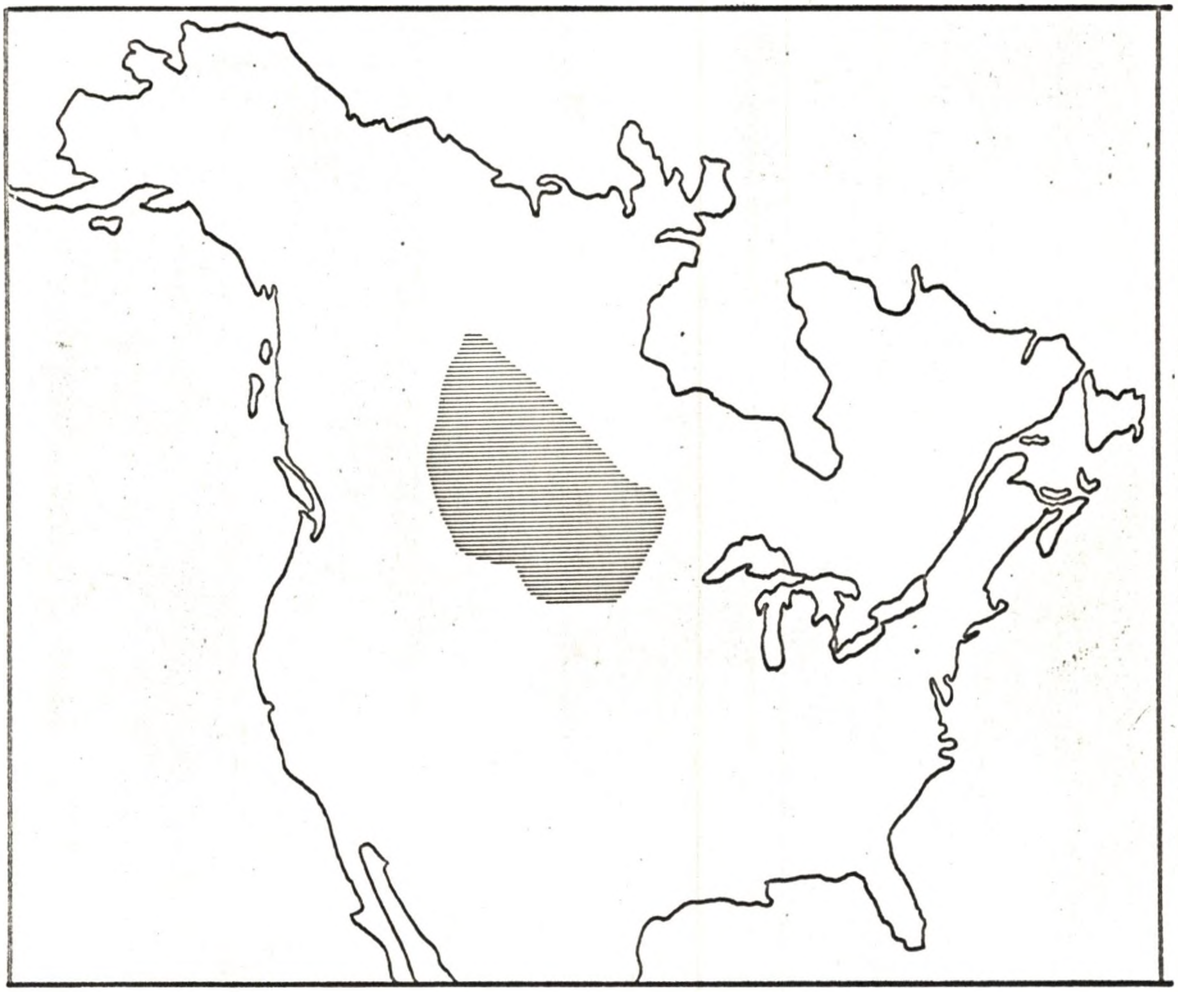


Fig. 1.--Distribution map of Bufo hemiophrys. Taken from Stebbins (1954) and Wright and Wright (1949).

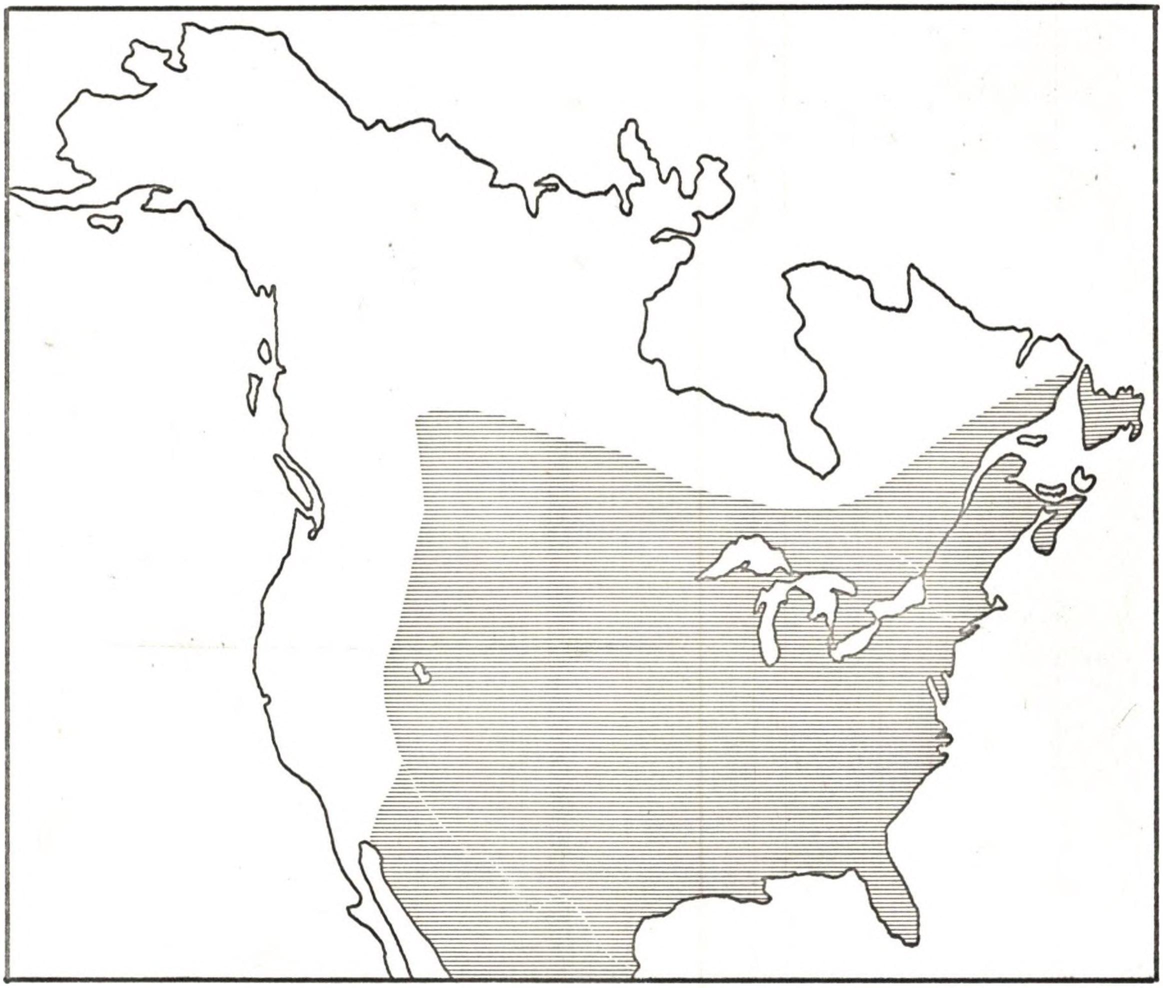


Fig. 2.--Distribution map of Rana pipiens. Taken from Stebbins (1954) and Wright and Wright (1949).

Fig. 3.--Distribution map of Rana septentrionalis. Taken from Wright and Wright (1949) and Breckenridge (1944).

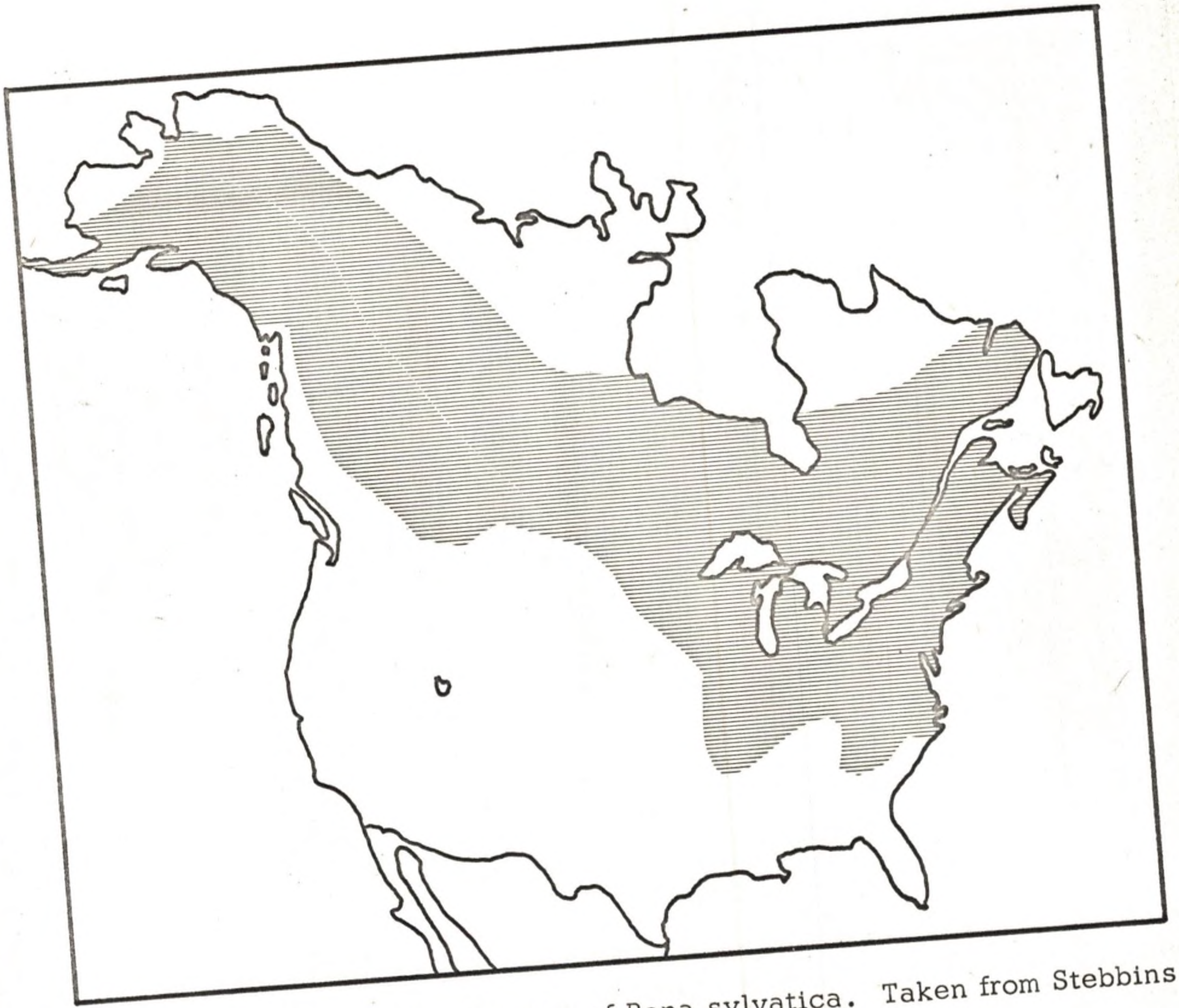


Fig. 4.--Distribution map of Rana sylvatica. Taken from Stebbins (1954) and Wright and Wright (1949).

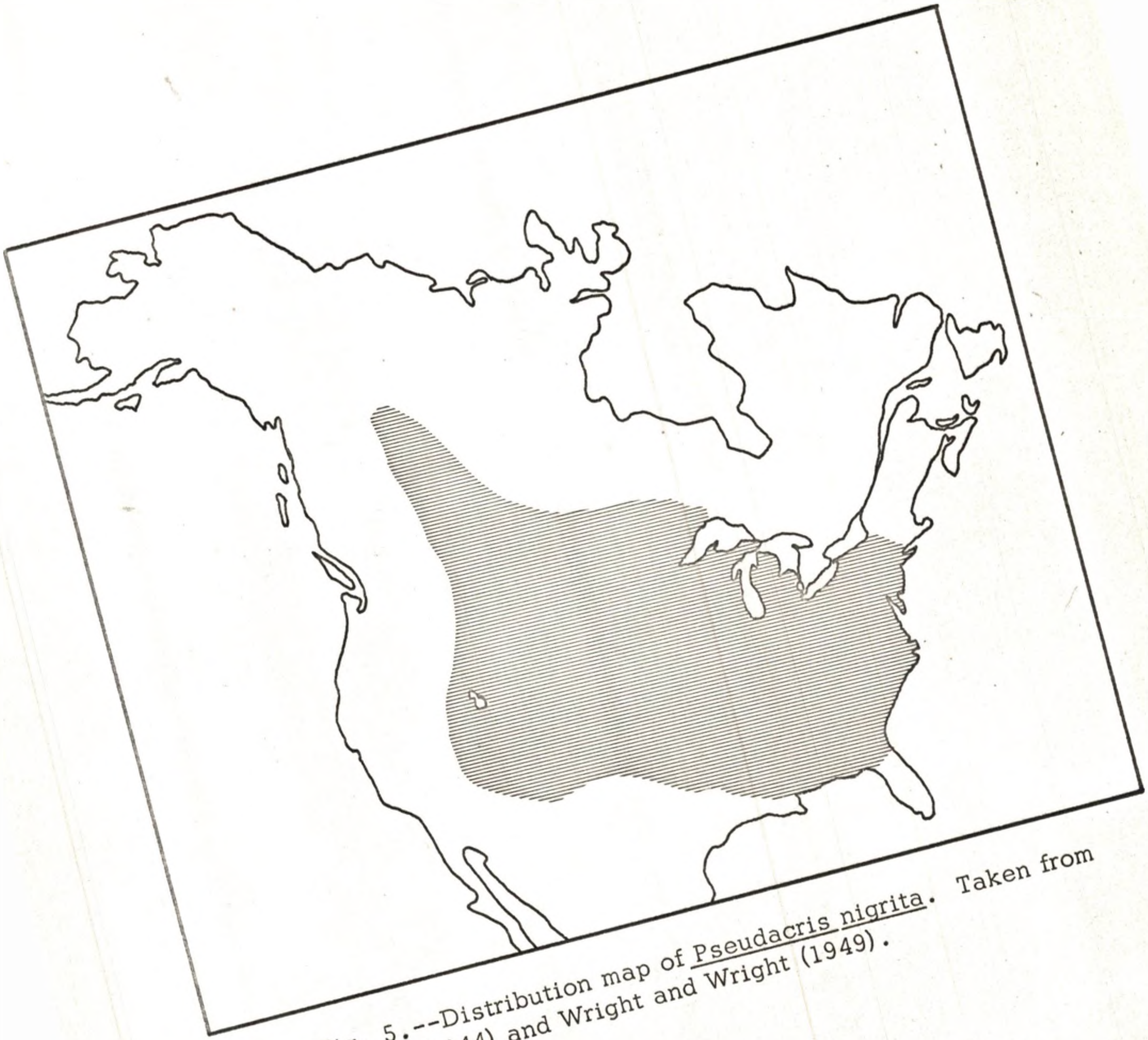


Fig. 5.--Distribution map of *Pseudacris nigrita*. Taken from Breckenridge (1944) and Wright and Wright (1949).

APPENDIX II

DATA TABLES

TABLE 1.--Data for *Bufo hemiophrys*

Test Temperature (^{o}C)	Sex (o) Female (+) Male	Body Weight (g)	Survival Time (minutes)	Log Survival Time
37.5	+	2.4	180+	2.2553+
37.5	+	1.2	180+	2.2553+
37.5	o	2.8	180+	2.2553+
37.5	o	1.4	180+	2.2553+
39.0	+	2.4	84	1.9243
39.0	+	4.8	40	1.6021
39.0	+	1.5	33	1.5185
39.0	o	3.1	34	1.5315
39.0	o	1.6	59	1.7709
39.0	o	1.9	25	1.3979
40.0	+	1.1	13	1.1139
40.0	+	3.2	8	0.9031
40.0	+	1.9	16	1.2041
40.0	o	2.4	6	0.7782
40.0	o	2.6	13	1.1139
40.0	o	2.3	9	0.9542

TABLE 2.--Data for *Rana pipiens*

Test Temperature (°C)	Sex (o) Female (+) Male	Body Weight (g)	Survival Time (minutes)	Log Survival Time
36.0	o	10.6	180+	2.2553+
36.0	o	8.8	180+	2.2553+
36.0	o	9.4	180+	2.2553+
36.0	o	9.7	180+	2.2553+
37.5	+	12.4	161	2.2068
37.5	+	6.6	174	2.2405
37.5	+	12.0	170	2.2304
37.5	o	7.6	180+	2.2553+
37.5	o	8.7	180+	2.2553+
37.5	o	9.1	132	2.1206
39.0	+	7.4	31	1.4914
39.0	+	9.2	5.0	0.6990
39.0	+	7.5	28	1.4472
39.0	o	7.3	5.8	0.7634
39.0	o	7.3	8.0	0.9031
39.0	o	9.2	16	1.2041
41.0	+	5.7	1.1	0.0414
41.0	+	9.6	2.0	0.3010
41.0	+	17.2	2.1	0.3222
41.0	o	10.1	2.0	0.3010
41.0	o	19.6	2.1	0.3222
41.0	o	41	3.6	0.5563

TABLE 3.--Data for *Rana septentrionalis*

Test Temperature (°C)	Sex (o) Female (+) Male	Body Weight (g)	Survival Time (minutes)	Log Survival Time
35.0	+	6.0	180+	2.2553+
35.0	+	8.5	180+	2.2553+
35.0	o	8.3	180+	2.2553+
35.0	o	17.0	180+	2.2553+
36.0	+	22.0	48	1.6812
36.0	+	7.0	108	2.0334
36.0	+	4.2	91	1.9590
36.0	+	5.3	27	1.4314
36.0	o	20.3	47	1.6721
36.0	o	4.6	27	1.4314
36,0	o	8.0	92	1.9638
36.0	o	10.0	111	2.0453
37.5	+	8.6	19	1.2788
37.5	+	6.0	20	1.3010
37.5	o	12.7	10	1.0000
37.5	o	10.1	10	1.0000
37.5	o	6.5	6	0.7782
37.5	o	6.9	5	0.6990
37.5	o	23.9	4	0.6021
37.5	o	18.2	7	0.8451
39.0	+	13.0	2.7	0.4314
39.0	+	16.0	3.3	0.5185
39.0	+	8.0	4.2	0.6232
39.0	o	17.9	3.7	0.5682
39.0	o	20.6	3.2	0.5051
39.0	o	10.0	2.1	0.3222
41.0	+	13.9	1.1	0.0414
41.0	o	17.3	1.3	0.1139
41.0	o	29.8	1.4	0.1461
41.0	o	19.3	1.5	0.1761
41.0	o	18.2	1.25	0.0969
41.0	o	16.0	1.1	0.0414

TABLE 4.--Data for *Rana sylvatica*

Test Temperature (°C)	Sex (o) Female (+) Male	Body Weight (g)	Survival Time (minutes)	Log Survival Time
34.0	+	5.1	180+	2.2553+
34.0	+	7.9	180+	2.2553+
34.0	o	6.5	180+	2.2553+
34.0	o	5.3	180+	2.2553+
35.0	+	7.5	70	1.8451
35.0	+	6.3	92	1.9638
35.0	+	6.7	60	1.7782
35.0	o	5.3	136	2.1335
35.0	o	2.6	48	1.6812
35.0	o	8.9	74	1.8692
36.0	+	6.6	31	1.4914
36.0	+	5.1	110	2.0414
36.0	+	1.4	60	1.7782
36.0	+	6.5	15	1.1761
36.0	+	7.8	53	1.7243
36.0	+	6.5	20	1.3010
36.0	o	18.1	29	1.4624
36.0	o	1.9	60	1.7782
36.0	o	1.3	8.0	0.9031
36.0	o	5.3	15	1.1761
36.0	o	6.7	73	1.8633
36.0	o	8.3	34	1.5315
37.5	+	4.7	5.0	0.6990
37.5	+	6.0	4.2	0.6232
37.5	+	5.3	7.9	0.8976
37.5	o	5.8	4.0	0.6021
37.5	o	5.0	7.5	0.8751
37.5	o	5.4	2.6	0.4150
39.0	+	1.8	0.75	-0.1249
39.0	+	9.1	0.8	-0.0969
39.0	+	6.8	1.4	0.1461
39.0	o	5.4	0.95	-0.0223
39.0	o	8.5	1.0	0.0000
39.0	o	3.1	0.5	-0.3010
41.0	+	2.0	0.2	-0.6990
41.0	+	3.7	0.35	-0.4559
41.0	+	3.4	0.25	-0.6021

(continued)

TABLE 4.--Continued

Test Temperature (°C)	Sex (o) Female (+) Male	Body Weight (g)	Survival Time (minutes)	Log Survival Time
41.0	o	14.5	0.75	-0.1249
41.0	o	2.9	0.8	-0.0969
41.0	o	1.4	0.3	-0.5229
41.0	o	2.8	0.35	-0.4559
41.0	0	3.5	0.65	-0.1871
41.0	o	2.7	0.4	-0.3979

TABLE 5.--Data for *Pseudacris nigrita*

Test Temperature (°C)	Sex (o) Female (+) Male	Body Weight (g)	Survival Time (minutes)	Log Survival Time
34.0	+	0.95	180+	2.2553+
34.0	+	0.75	180+	2.2553+
34.0	o	1.0	180+	2.2553+
34.0	o	0.60	180+	2.2553+
35.0	+	0.62	180+	2.2553+
35.0	+	0.65	11.5	1.0607
35.0	+	0.75	45	1.6532
35.0	+	0.70	50	1.6990
35.0	+	0.90	55	1.7404
35.0	+	0.60	165	2.2175
35.0	o	0.90	35	1.5441
35.0	0	0.60	176	2.2455
35.0	o	0.65	42	1.6232
35.0	0	0.61	125	2.0969
36.0	+	0.70	45	1.6532
36.0	+	0.85	8.0	0.9031
36.0	+	1.25	10.5	1.0212

(continued)

TABLE 5.--Continued

Test Temperature (°C)	Sex (o) Female (+) Male	Body Weight (g)	Survival Time (minutes)	Log Survival Time
36.0	+	0.91	180+	2.2553+
36.0	o	0.96	69	1.8388
36.0	0	1.10	61	1.7853
36.0	0	0.61	9.5	0.9777
36.0	o	0.96	73	1.8633
36.0	o	0.90	13	1.1139
37.5	+	0.52	4.5	0.6532
37.5	+	0.65	5.5	0.7404
37.5	+	0.80	5.0	0.6990
37.5	+	0.77	8.0	0.9031
37.5	+	0.85	6.5	0.8129
37.5	+	0.96	6.5	0.8129
37.5	o	0.25	18.5	1.2672
37.5	o	0.35	6.0	0.7782
37.5	o	0.30	21.0	1.3222
37.5	o	0.70	5.3	0.7243
37.5	o	0.70	8.3	0.9191
37.5	o	0.50	7.5	0.8751
37.5	o	0.67	7.0	0.8461
39.0	+	0.53	2.6	0.4150
39.0	+	0.82	1.3	0.1139
39.0	+	0.80	2.2	0.3424
39.0	o	0.7	5.5	0.7404
39.0	o	0.6	1.5	0.1761
39.0	o	0.5	1.5	0.1761
39.0	o	0.6	4.0	0.6021
39.0	o	0.7	2.0	0.3010

APPENDIX III

SAMPLE CALCULATIONS

LD_{50} Calculation by the Spearman-Kärber Method

x_i	Dose (temperature in °C)
n_i	Sample size
r_i	Number that die in less than 60 minutes at dose x_i
p_i	r_i / n_i (response rate at dose x_i)
m	Median effective temperature dose at 60 minutes (the value of x for which $p=0.5$)

x_i	ni	r_i	p_i
34	4	0	0.00
35	6	1.5	0.25
36	12	9	0.75
37.5	6	6	1.00

<u>Rana sylvatica</u>

$$m = \sum \left[(p_{i+1} - p_i) \frac{(x_i + x_{i+1})}{2} \right]$$

$$m = 0.25 \frac{69}{2} + 0.5 \frac{71}{2} + 0.25 \frac{73.5}{2}$$

$$m = 35.56^{o}C$$

LD_{50} Calculation by the Reed-Muench Method

x_i	Dose (temperature in °C)
n	Sample size
r_i	Number that die in less than 60 minutes at dose x_i
d	Distance between test doses in °C
S_-	Sum of all values of r for this and lower doses
S_+	Sum of all values of n-r for this and higher doses
m	An interpolation between doses x_i and x_{i+1}

		x	n*	r	n-r	S_-	S_+
		34	6	0	6	0	12
m	x_i	35	6	1.5	4.5	1.5	6
	x_{i+1}	36	6	4.5	1.5	6	1.5
		37.5	6	6	0	12	0

*Sample sizes were adjusted to equal 6 ; r values were adjusted proportionately.

$$m = x_i + \frac{d(S_+ - S_-)}{n - r_i + r_{i+1}}$$

$$m = 35 + \frac{1(6 - 1.5)}{6 - 1.5 + 4.5}$$

$$m = 35.5^{\circ}C$$

LD_{50} calculation by the Dragstedt-Behrens Method

x	dose (temperature in °C)
n	sample size
r_i	Number that die in less than 60 minutes at dose x_i
S_-	Sum of all values of r for this and lower doses
S_+	Sum of all values of $n-r$ for this and higher doses

$$p = \frac{S_-}{S_- + S_+}$$

m is obtained by linear interpolation between two successive values of p on either side of 0.50

		x	n*	r	n-r	S_-	S_+	p
		34	6	0	6	0	12	0
m	x_i	35	6	1.5	4.5	1.5	6	.2
	x_{i+1}	36	6	4.5	1.5	6	1.5	.8
		37.5	6	6	0	12	0	1.0

*Sample sizes were adjusted to equal 6; r values were adjusted proportionately.

$$m = x_i + \frac{0.5 - p_i}{p_{i+1} - p_i}$$

$$m = 35 + \frac{0.5 - 0.2}{0.8 - 0.2}$$

$$m = 35.5^{\circ}C$$

LD_{50} Calculation by the Thompson Moving Average Method

x Dose (temperature in °C)
d Distance between doses in °C
k Span of the moving average

$$p = \frac{S_-}{S_- + S_+}$$

$$m = x_i + \frac{d(k+1)}{2} - df$$

where

$$f = \frac{(p_{i+1} + p_{i+2} + \cdots + p_{i+k} - \frac{k}{2})}{p_{i+k} - p_i}$$

$$f = \frac{0.8 + 1.0 - 2/2}{1.0 - 0.2} = 1.0$$

$$m = 35 + \frac{(1)(2+1)}{2} - 1.0\,(1.0)$$

$$m = 35.5^{\circ}\mathrm{C}$$

LD_{50} Calculation by the Gaddum Method of Extreme Effective Doses

x	Series											
(°C)	1	2	3	4	5	6	7	8	9	10	11	12
34	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	-	+	+	-	-
36	-	+	+	-	-	-	-	-	-	-	+	-
37.5	-	-	-	-	-	-	-	-	-	-	-	-
m	35.5	35.75	36.75	35.5	35.5	35.5	35.5	34.5	35.5	35.5	35.5	34.5

+ Indicates survival

\- Indicates death

m is the mean of the lowest effective and the highest ineffective doses.

$$m = \left[2(36.75) + 2(34.5) + 8(35.5)\right] \div 12$$

$$m = 35.52^{\circ}C$$

LITERATURE CITED

Breckenridge, W. J. 1944. Reptiles and amphibians of Minnesota. Univ. of Minnesota Press, Minneapolis, Minn. 202p.

Cowles, R.B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Am. Mus. Nat. Hist. 83 : 261-296.

Dawson, W. R. 1960. Physiological responses to temperature in the lizard. Physiol. Zool. 33: 87-103.

Dill, D. B. 1938. Life, heat and altitude. Harvard Univ. Press, Cambridge, Mass. 211p.

Finney, D. J. 1952. Statistical method in biological assay. Hafner Publ. Co., New York. 661p.

Heatwole, H., M. Mercado and E. Ortiz. 1965. Comparison of critical thermal maxima in two species of Puerto Rican frogs of the genus Eleutherodactylus. Physiol. Zool. 38 : 1-8.

Licht, Paul. 1964a. A comparative study of the thermal dependence of contractility in saurian skeletal muscle. Comp. Biochem. Physiol. 13:27-34.

Licht, Paul. 1964b. The temperature dependence of myosin-adenosinetriphosphatase and alkaline phosphatase in lizards. Comp. Biochem. Physiol. 12:331-340.

Marshall, W. H., and M. K. Buell. 1955. A study of the occurrence of amphibians in relation to a bog succession, Itasca Park, Minnesota. Ecology 36:381-387.

Odum, E. P. 1959. Fundamentals of ecology. W. B. Saunders Co., Philadelphia, Penn. 546p.

Oliver, J. A. 1955. North American amphibians and reptiles. Van Nostrand Co., Princeton, New Jersey. 359p.

Schmid, W. D. 1965a. High temperature tolerances of *Bufo hemiophrys* and *Bufo cognatus*. Ecology 46:559-560.

Schmid, W. D. 1965b. Some aspects of the water economies of nine species of amphibians. Ecology 46:261-269.

Stebbins, R. C. 1954. Amphibians and reptiles of western North America. McGraw-Hill Book Co., New York. 528p.

Ushakov, B. P. 1959. Heat resistance of tissues as a specific character of poikilothermic animals (in Russian, English summary). Zool. Zh. 38:1292-1302.

Ushakov, B. P. and I. S. Darevskii, 1960. Comparison of the heat resistance of muscle fibers and behavior toward temperature in two sympatric species of semi-desert lizards. Dokl.-Biol. Sci. Sect., English Transl. 128:770-773.

Wright, A. H., and A. A. Wright. 1949. Handbook of the frogs and toads of the United States and Canada. Comstock Publishing Assoc. 640p.