

A STUDY OF TEMPERATURE ACCLIMATION IN THE
SALAMANDERS PLETHODON DUNNI BISHOP,
PLETHODON VEHICULUM COOPER, AND ANEIDES FERREUS COPE

by

OLIVER WILLIAM JOHNSON

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APPROVED:

Redacted for Privacy

Associate Professor of Zoology

In Charge of Major

¹²
Redacted for Privacy

Chairman of ~~Department~~ of Zoology

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

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Typed by Ruth Chadwick

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INTRODUCTION

The physical factors of the natural environment important to living organisms are temperature, moisture, pressures and currents, light, atmospheric gases, salts, and soil. These factors are important in controlling the actual distribution and abundance of organisms in nature (22, p. 35). The effects of temperature on animals and plants have received a great deal of attention from biologists, probably because of the relative ease with which temperature is measured. Belehradek states that well over 3000 papers have appeared, touching on various effects of temperature on organisms, since his monographic review in 1935 (1, p. 59).

The existence of thermal adjustment and changes in metabolism as a result of a change in the thermal environment is a well-established phenomenon in nature. There are essentially three levels on which the organism achieves adjustment or regulation as the environment changes. At the most immediate level there may be day-to-day changes in its systematic and cellular organization in direct response to changes in temperature (i.e., acclimation). Next there may be long-term responses operating throughout the life of the individual (i.e.,

acclimatization). These effects of the thermal history may be coupled with the results of the conflicting or reinforcing influences of other environmental factors such as the photoperiod, salinity or humidity. Finally, the process of compensation must be considered on the phylogenetic level, at which the mechanisms for the production of direct responses to temperature and for the anticipation of seasonal changes have been fixed in the heritage of the species by selection (i.e., adaptation). (11, p. 208)

The demonstration of acclimation and a measure of the amount of acclimation have been shown quantitatively in two ways: first, by the use of survival times at various upper and/or lower lethal temperatures when the organism has been exposed previously to different thermal levels (i.e., acclimated to some prior temperature) and/or second, by the alteration of some metabolic function or rate process (heart beat, breathing rate, oxygen consumption, etc.).

Quantitative studies of acclimation as a factor in the resistance of various animals to high and low temperatures have been repeatedly undertaken. In poikilotherms, acclimation has been reported for bacteria, protozoa, coelenterates, crustaceans, molluscs, insects, fish, turtles, reptiles, and amphibians. Any attempt on the

part of the author to review critically the literature on the thermal aspects of poikilotherms alone would be a staggering task, and a great deal of reliance has been placed on some excellent reviews of the literature on this topic.

The best general reference that has reviewed and summarized the older literature is that of C. B. Davenport (7, p. 219-273). Heilbrunn, in his text, has devoted a chapter to the topic of acclimatization (14, p. 546-563), and Prosser et al. has briefly reviewed the effect of temperature and acclimation on the metabolic aspects of poikilotherms (24, p. 346-361). A review of the literature to show intra- and inter-specific variation by the use of environmental stresses, of which one was temperature, in order to elucidate the nature of the species was made by Prosser (23, p. 238-244). Bullock has published a review of the literature clarifying the phenomenon of temperature compensation by poikilotherms as measured by various rate processes of metabolism and activity, and emphasizes the topic of seasonal and experimental acclimation (4, p. 317-321). In a very recent publication, F. E. J. Fry (11, p. 207-224) brings the excellent review of Bullock (vide supra) up to date on the topic of temperature compensation and briefly discusses acclimation, acclimatization, and adaptation. Various specialized

reviews of temperature effects have been published, such as Rao and Bullock's paper showing that Q_{10} is a function of size and that poikilotherms exhibit compensatory mechanisms to adapt themselves to habitats of different temperatures (25, p. 33-44). The physiological aspects of heat and cold with respect to rate processes (temperature coefficients, thermal optima and maxima, and heat and cold injury) and thermal adaptation have been reviewed extensively by Belehradek (1, p. 59-82).

Within the vertebrates, all aspects of acclimation, especially in the case of reptiles and amphibians, are little known, and its significance in evolution or even contemporary survival has not been appraised. In fact, in the entire class of Amphibia, little is known about lethal temperature limits. A large amount of valuable work in experimental acclimation of fresh-water fish has been shown in recent years, especially by Fry and his collaborators in Toronto, and has provided excellent data on the extent and rate of acclimation in many species.

In their research on tadpoles of Bufo terrestris, Davenport and Castle (7, p. 253-254) demonstrated a change in the upper lethal temperatures depending upon the temperatures at which they were acclimated. The authors showed that groups raised at 15° and 25° C. had average upper lethal temperatures of 40.3° and 43.5° C.

respectively.* The difference in the upper lethal temperature was lost when readapted to the alternative temperature, but this loss was not rapid, the upper lethal temperature of the 25° group being 41.5° after 17 days reacclimation at 15°. Hathaway, working with tadpoles of Bufo terrestris americanus, was able to show the same phenomenon; that is, the lower the acclimation temperature is, the lower the upper lethal temperature is (12, p. 187).

Mellanby (16, p. 28) has shown low temperature acclimation to exist in Rana temporaria and Salamandra salamandra. In his work, adults that had been acclimated to 10° and 30° were transferred to a 0° environment. The high acclimated group (30°) proved to be inactive at 0°, but the other, active. In addition, Mellanby indicated that the length of exposure is an important factor in temperature acclimation, and this has been extensively investigated in fishes by Doudoroff (8, p. 232-238) and Brett (2, p. 34-39) (3, p. 5-28).

A number of papers relate geographic distribution of some amphibians, notably anurans, to rates of development, temperature tolerances, etc. (17, p. 459-478) (18, p. 189-213) (19, p. 375-388) (20, p. 345-370) (21, p. 1-24) (30, p. 344-354) (31, p. 164-176). These researches are

* All temperatures cited in this thesis are in degrees centigrade.

not concerned with the individual adaptation of the organism, but deal with reactions of eggs and their development to temperature. They reveal that the limits of temperature tolerance and rates of development are correlated with the geographical distribution and breeding habits. The results indicate that such differences as found are on a genetic level and probably involve physiological races.

McFarland (15, p. 191-194) has clearly shown acclimation in the California newt, Taricha torosa, with respect to upper lethal temperatures. He acclimated three groups of salamanders to 10°, 22°, and 30° and exposed each group to lethal temperatures of 33.5°, 36.0°, and 38.0° respectively. He reported that the group acclimated to 10° could withstand a lethal temperature of 36° for six and three-fourths minutes, whereas the 30°-acclimated group could withstand this lethal temperature for 140 minutes.

Zweifel (32, p. 64-69), working at or near the Mountain Lake Biological Station of the University of Virginia, has studied the critical thermal maxima of five plethodontid salamanders (Desmognathus quadrimaculatus, Desmognathus f. fuscus, Desmognathus ochrophaeus carolinensis, Eurycea b. bislineata, and Pseudotriton r. ruber) and a species of salamandrid (Diemictylus v. virescens) and the effect of acclimation on the critical thermal maxima. The critical thermal maximum (CTM) has

been defined by Cowles and Bogert (6, p. 277) as a point at which locomotor activity becomes disorganized and the animal is incapable of escaping from conditions that, if continued, will lead to its death. This thermal level represents an ecological, not a physiological, lethal temperature. The results of Zweifel that are pertinent to the present investigation may best be presented in the form of a table:

Species	Number of Individuals	Acclimation time (days)	CTM (°C.)	
			Mean	Range
D. q. D. f. f. D. v. v.	5° C. acclimation			
	10	6 - 10	30.1 \pm 0.12	29.5 - 30.5
	9	6 - 9	31.0 \pm 0.17	30.0 - 31.5
	1	6	34.5	
	15° C. acclimation			
	8	6	31.4 \pm 0.10	31.0 - 32.0
	10	6	32.2 \pm 0.13	31.5 - 32.5
	room temperature acclimation (17° - 26° C.)			
	19	2	31.9 \pm 0.10	31.5 - 32.5
	8	5	33.5 \pm 0.19	33.0 - 34.0
D. o. c.	18	5	31.5 \pm 0.14	30.5 - 32.0
E. b. b.	1	several	35.0	
P. r. r.	1	2	34.5	
D. v. v.	10	5	37.3 \pm 0.20	36.0 - 38.0

It may be seen from an inspection of the table above that acclimation occurs in two species of Desmognathus (D. q. and D. f. f.), comparing the 5 $^{\circ}$ and 15 $^{\circ}$ acclimation temperatures, and that the salamandrid Desmognathus (D. v. v.)

as far as the experiment went, may also have acclimated.

The only records in the literature on salamanders at all comparable to Zweifel's work is that of Stebbins on Ensatina eschscholtzi (27, p. 107-108) in which the CTM was recorded between 32° and 34°, and Dumas (9, p. 488) who recorded time to death at 26°, 28°, 32° and 36° for Plethodon dunni and Plethodon vehiculum. By extrapolation of Dumas's data, the CTM was found to be approximately at 32° for both species. The thermal histories of the experimental animals prior to the experiments were not determined by either Stebbins or Dumas, and, as Fry has emphatically pointed out (10, p. 7), the thermal history of the organism must always be taken into account before any measurement of the organism as a whole will have a precise meaning.

Because of the relative scarcity of research on temperature acclimation in salamanders, it was decided to determine the extent of acclimation that may be induced experimentally in three species of plethodontid salamanders: Plethodon dunni Bishop, Plethodon vehiculum Cooper, and Aneides ferreus Cope. These three species were selected for several reasons: (1) they may be obtained locally, (2) they are among the most terrestrial of salamanders (most temperature acclimation reported has been done with aquatic species of invertebrates and

vertebrates), (3) they are sympatric over much of their range in Oregon (see Plate I), (4) they differ in size (see Plate II), and (5) these three species may occupy the same ecosystem.

The last reason cited above, that these three species may occupy the same ecosystem, requires a note of qualification. The ecology of Plethodon vehiculum and Plethodon dunni has been studied by Dumas (9, p. 484-495) who states on page 494 that both species occupy the Rocky Outcrop-Talus Slope Ecosystem; however, P. vehiculum is more often found in the less moist areas of the habitat (which implies higher niche temperature environments), and P. dunni inhabits the wetter substrates and is more tolerant of low temperatures. Dumas further states (9, p. 486) that Aneides ferreus occurs most frequently in the more xeric portions fringing this ecosystem. It is also reported that A. ferreus is often found in rotting fir logs and stumps in open clearings (28, p. 60). Hence, although the three species may occupy the same ecosystem and possess sympatric distributions in Oregon, their microhabitat preferences may be different, and A. ferreus may often be found out of the Rocky Outcrop-Talus Slope Ecosystem in a more xeric habitat.

PLATE I

Map 1

Distribution of *Plethodon dunni*

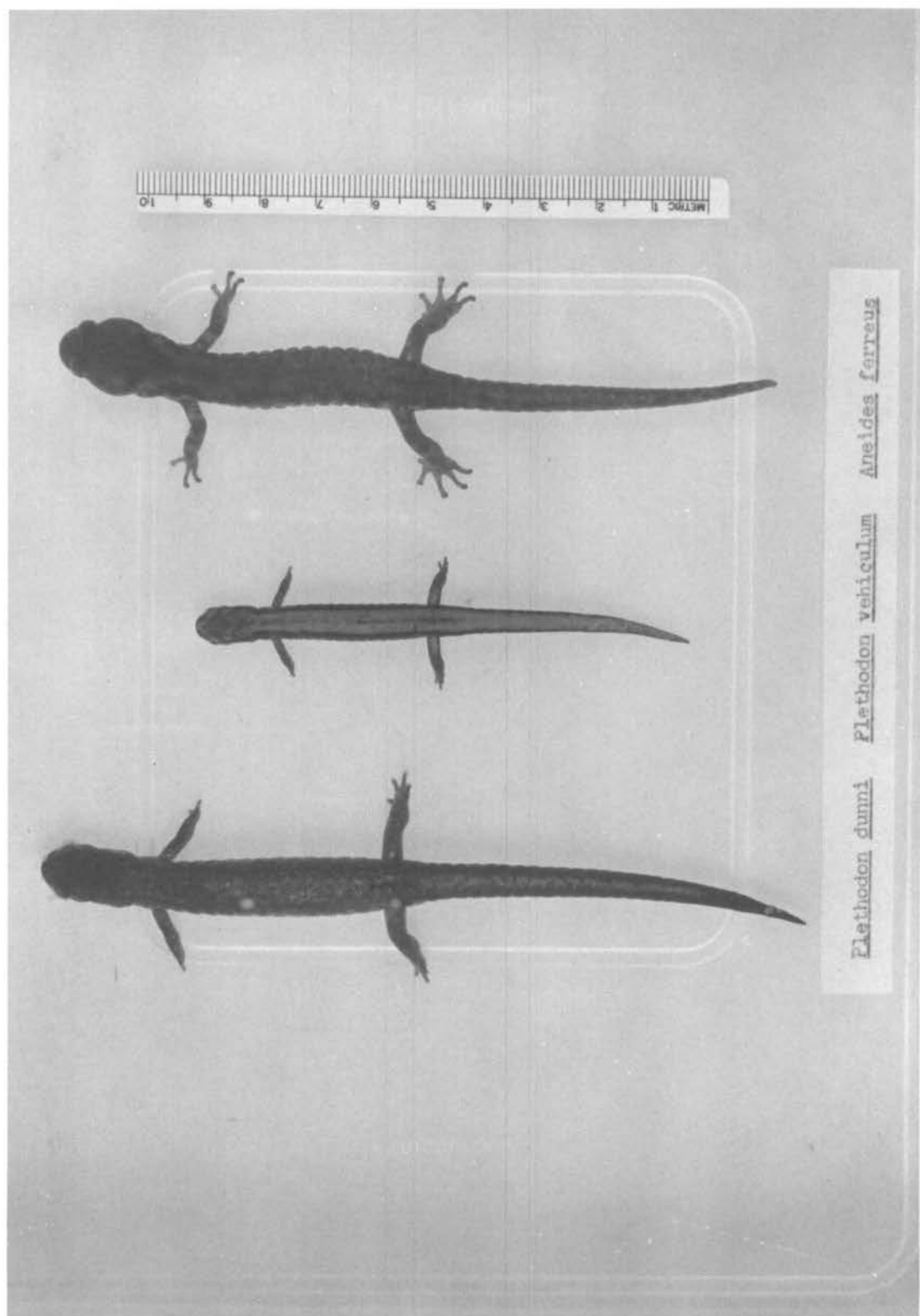
Map 2

Distribution of *Plethodon vehiculum*

Map 3

Distribution of *Aneides ferreus*

PLATE II



METHODS AND MATERIALS

The three species of plethodontid salamanders used for this study (Plethodon vehiculum Cooper, Plethodon dunni Bishop, and Aneides ferreus Cope) were collected from Benton, Linn, Lane and Lincoln Counties in Oregon during different months of the years 1956, 1957 and 1958. The specific collection sites are illustrated on Map 4. The habitat utilized for the collections was the Rocky Outcrop-Talus Slope Ecosystem described by Dumas (9, p. 485). A small number of Aneides ferreus were collected in rotting Douglas fir (Pseudotsuga taxifolia) logs and stumps. The salamanders were brought to the laboratory, rinsed thoroughly with tap water, and separated into size groups. The adult or near-adult specimens were placed in quart jars containing moist paper toweling and kept in a refrigerator that maintained a temperature of $6^{\circ} \pm 0.75^{\circ}$ until the acclimation chamber or water bath was available for use.

The period of time used to acclimate the animals to a particular thermal level was arbitrarily selected at seven days. This time interval was felt to be adequate on the basis of the researches of Doudoroff (8, p. 235) and Brett (2, p. 34) (3, p. 5-28). These authors, working principally with different species of fresh-water and

marine fishes, found the major portion of acclimation to have taken place in less than seven days. For example, Brett (2, p. 34) found that within 24 hours bullheads, Ameiurus nebulosus, were completely acclimated to a temperature of 20° using a test-lethal temperature of 35.5°. Further, Sumner and Doudoroff (29, p. 417) found the greater part of acclimation temperature to be complete within 24 hours for the long-jawed goby, Gillichthys mirabilis, a marine species of fish. In addition, to be consistent with the only other comparable investigation in salamanders, seven days acclimation time was used for comparison with the paper of McFarland (15, p. 192) who used one week as his acclimation time for the California newt, Taricha torosa.

Four acclimation temperatures were initially attempted: 6°, 12°, 15°, and 18°. Repeated attempts to acclimate the three species to a thermal level of 18° proved fruitless. In one instance, however, Aneides ferreus was successfully acclimated to 18°. After several additional attempts, the 18° acclimation temperature was discontinued for all three species. The acclimation apparatuses were thermostatically-controlled water baths and refrigerators that could be maintained at the acclimation temperature to within 0.5° or less, except for the 6° acclimation box, which could be maintained to within

0.75°. The salamanders were not fed during the period of acclimation or during the experiments.

At the end of the acclimation period the specimens in each test group were carefully dried with paper toweling and weighed to the nearest tenth of a gram on a Welch Double-Beam Balance. The salamanders of each test group were then placed, one to a flask (Figure 1, C), in the lethal temperature apparatus and the time to 50 percent mortality was recorded.

The lethal temperature apparatus, shown in Figure 1, is essentially a thermostatically controlled water bath with 200-ml. flasks immersed in the water. The temperature of the water bath was maintained at the desired temperature to within 0.5° or less. The lethal temperature apparatus was permitted to come to an equilibrium for a period of 60 minutes before each experiment was begun. The flasks were lined with moist paper toweling and covered with squares of plate glass to permit continuous observation of the salamanders being subjected to the lethal temperature and to maintain an atmosphere saturated with water vapor within the flasks. The general reactions of the salamanders at the various lethal test temperatures were observed and recorded.

Fry et al. (12, p. 9) defined the upper limit of thermal tolerance, for a given acclimation level, as the

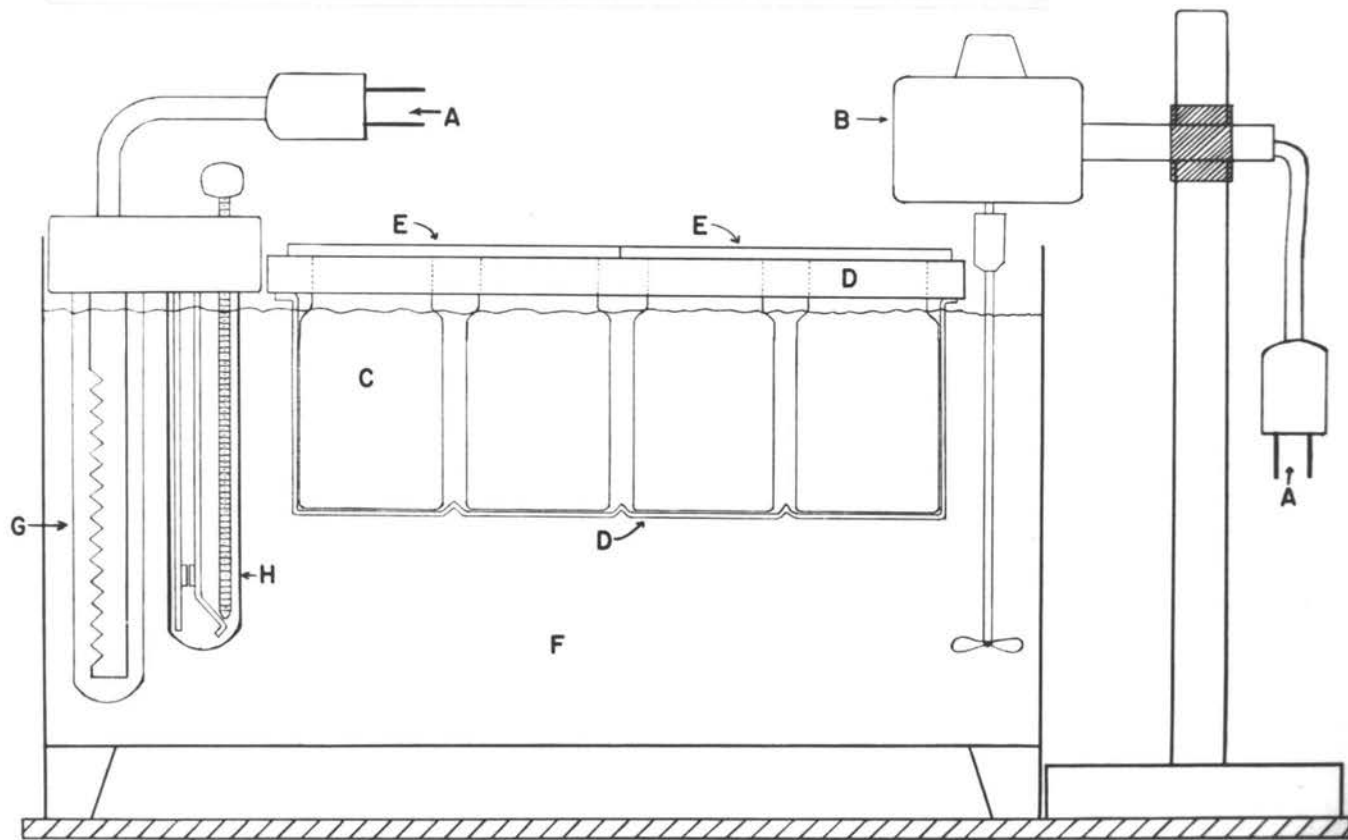
incipient lethal temperature. The incipient lethal temperature is that temperature beyond which 50 percent of the population can no longer live for an indefinite time. The method used by Brett (2, p. 5) to determine the incipient lethal temperature for fish was the temperature at which 50 percent of the fish die if exposed for a period of twelve hours. In this study, the incipient lethal temperature for each acclimation level was determined by exposing the salamanders to a lethal test temperature of 38° and reducing the test temperature at 3° intervals for each test group until a temperature level was obtained at which 50 percent of the test group would survive for a period of twelve hours or longer. It was found necessary to use the test temperatures of 38°, 35°, 32°, 29°, and 27° for Plethodon vehiculum and Plethodon dunni. Aneides ferreus was found to have a higher thermal tolerance experimentally, and the lethal test temperatures of 38°, 35° and 32° were used.

The salamanders were observed at frequent intervals during each experiment to determine accurately the time to death for 50 percent of the test animals. The criterion used to determine the death of the salamanders was the cessation of the heart contraction. The heart could be observed easily through the rather transparent tissues on the ventral side of the salamander in the pectoral

region. It was found, experimentally, that if the animal was removed from the lethal temperature apparatus before the heart ceased to contract, the salamander would recover if its body temperature was reduced to a non-lethal level (e.g., 6°). Repeated trials showed that lowering the body temperature to a non-lethal level for as long as 72 hours after the heart contractions had ceased would not revive them.

At the conclusion of each experiment, the specimens were re-weighed to the nearest tenth of a gram to determine any weight changes. The snout to the posterior margin of the vent (cloacal opening) and the total length measurements were taken, and the specimens were then labelled with an identifying number and preserved in 70 percent isopropyl alcohol.

LETHAL TEMPERATURE APPARATUS



A. plug to 110 V. a.c.
 B. rheostat controlled stirrer
 C. 150ml. wide mouth bottles
 D. bottle support rack

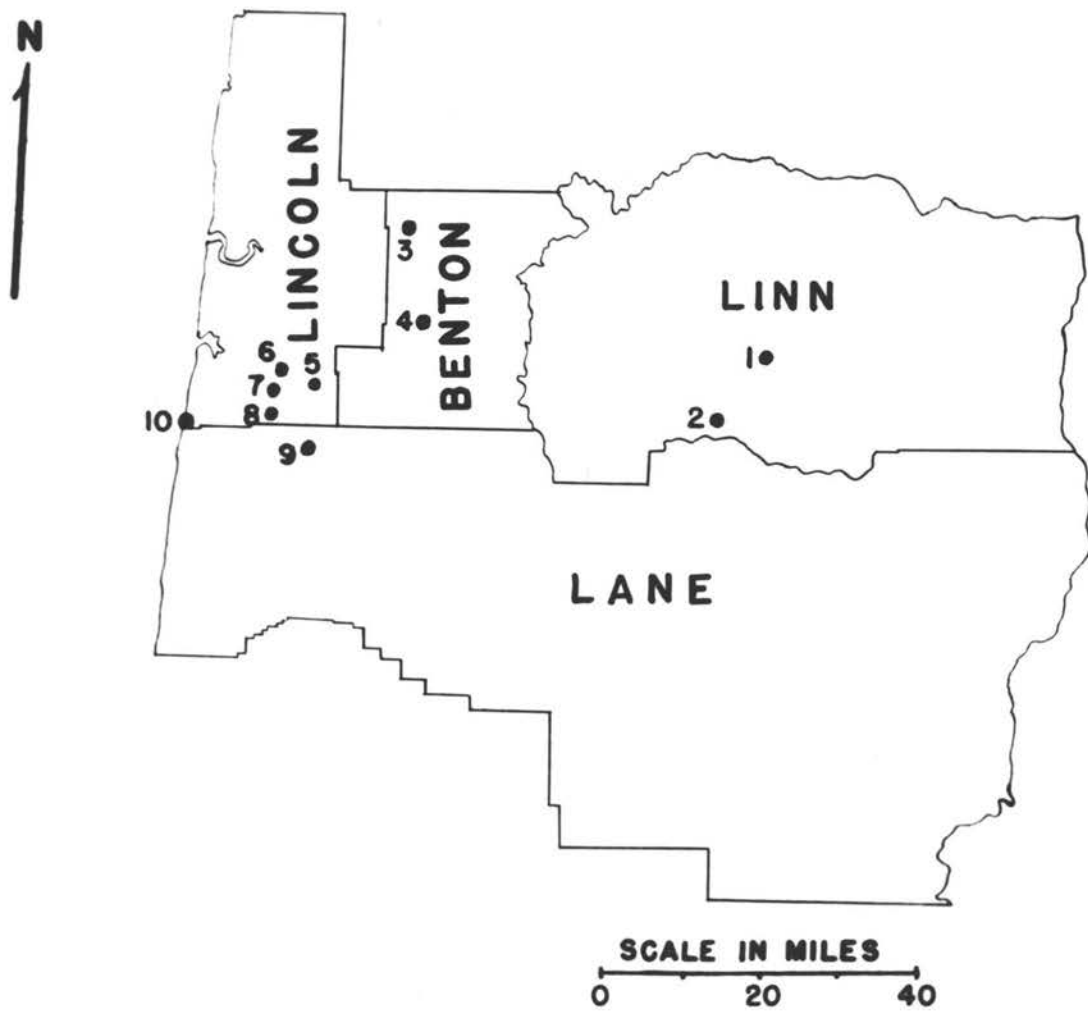
E. glass cover
 F. water bath
 G. heater
 H. thermostat

Figure 1

MAP 4

Collection Area and Species Collected

1. Six miles northeast of Foster, Linn County, Oregon; Aneides ferreus.
2. Five miles southeast of Holley on the Calapooya River road, Lane County, Oregon; Aneides ferreus.
3. One and one-half miles east of Summit at the headwaters of the Mary's River, Benton County, Oregon; Aneides ferreus and Plethodon vehiculum.
4. Eleven miles southwest of Philomath on Hyde and Well's Creek, Benton County, Oregon; Plethodon dunni and Plethodon vehiculum.
5. Sixteen miles west of Alsea on Oregon Highway 34, Lincoln County, Oregon; Plethodon dunni and Plethodon vehiculum.
6. Two miles south of Grass Mountain, Lincoln County, Oregon; Plethodon dunni and Plethodon vehiculum.
7. Twenty miles west of Alsea on Oregon Highway 34, Lincoln County, Oregon; Plethodon dunni and Plethodon vehiculum.
8. Two miles north of Fisher, Lincoln County, Oregon; Plethodon dunni and Plethodon vehiculum.
9. Eight miles southeast of Fisher on Wildcat Mountain, Lane County, Oregon; Aneides ferreus, Plethodon dunni and Plethodon vehiculum.
10. Cape Perpetua State Park, Lincoln County, Oregon; Aneides ferreus, Plethodon dunni and Plethodon vehiculum.



MAP 4

RESULTS

Behavioral Reaction to the Lethal Temperatures

The behavioral reaction of the three species studied varied somewhat, but merely in degree. The behavior of the variously acclimated groups when introduced into the different upper lethal test temperatures indicated that the behavioral sequence of succumbing was the same or nearly so. This tends to indicate that the cause of the heat death at the various lethal levels was the same.

The reaction to the extreme upper lethal test temperatures of 38° and 35° was quite marked and similar for all three species. The specimens were not tempered at an intermediate temperature before testing at 38° and 35° so that there was a lag in the response until their body temperature approached that of the flask. The behavioral and gross external physiological reactions were, in order of appearance: searching movements around the flasks, increased integumental mucus secretion, very rapid movements around the flask, violent convulsive movements, convulsive shudders with a pronounced arching of the back, milky exudate from the tail glands, and finally rigor. The specimens were characterized at death by hemorrhage of the lips and/or toes, dark masses of clotted blood

in the heart chambers, eyes withdrawn into their sockets, and a distended gular region.

Exposure to the moderate upper lethal temperatures of 32°, 29°, and 24° produced somewhat the same behavioral pattern as described above. The reaction was, however, of lessened intensity. Very often the specimens would show searching movements at first and then settle quietly at the bottom of the flask and remain quiet until succumbing to heat death. At other times the animals would climb up the sides of the flask and press their bodies against the glass cover until heat prostration caused them to release their grip and fall over backwards to the bottom of the flask. At this stage they usually lost their righting reflex.

The following points of difference characterized the death of these animals at moderate upper lethal temperatures: eyes not withdrawn into their sockets, absence of observable blood in the heart chambers, and lack of pronounced body rigor. Further, the heartbeat was very faint and rapid as the animal approached heat death, and it then became very slow and faint until it stopped altogether. It was observed also that if the animal was cooled to a lower temperature of 6° while the heart was still beating, the specimen would survive regardless of the lethal temperature, even though it may have been quite close to the

death point. The time of recovery, however, was very long for those specimens which were quite close to heat death.

Lethal Temperature Results

Plethodon dunni

The results of the experiments on the upper lethal temperatures of Plethodon dunni as a function of acclimation are given in Table I. The data of Table I are graphically summarized in Figure 2. Figure 2 shows that from 6° to 15° acclimation there is no marked increase in survival time at each lethal temperature. Reference to Figure 2 also shows that the lethal temperature-50 percent survival time curves have two major inflections, one at 35° and the other at 29° for the three acclimation levels tested.

The extent of the change in the slope at these two major inflections in Figure 2 may be shown more clearly in Table II, since the curves in Figure 2 are plotted semi-logarithmically, and this has the effect of shortening the arithmetic scale of the longer survival times at the lower lethal temperatures.

For the purpose of emphasizing the marked gain in survival time between the different lethal temperatures, Table II was prepared by recalculating the data from Table

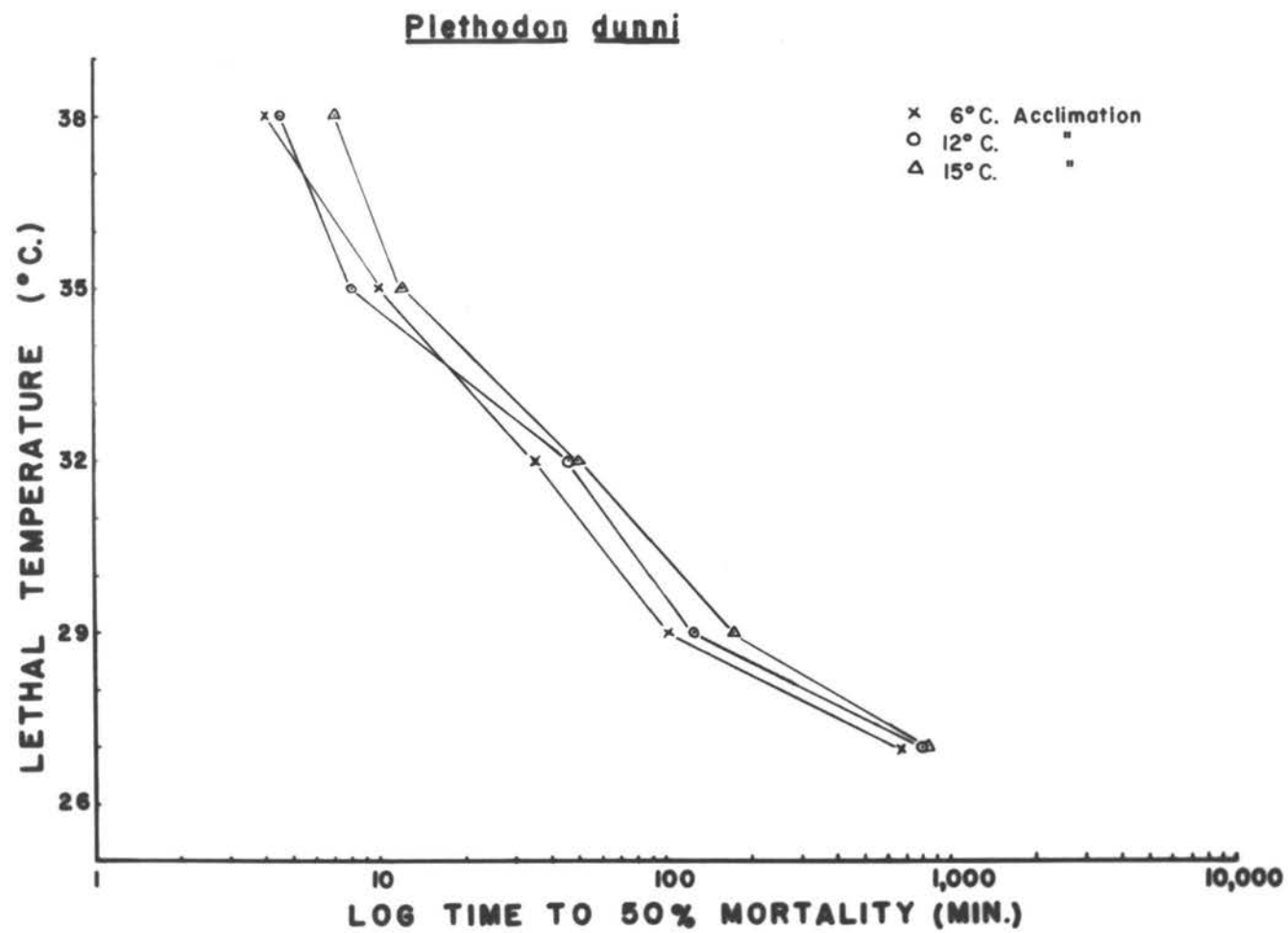


Figure 2

Table I. Data summary of acclimation - lethal temperature experiments on Plethodon dunni

Acclim. Temp.	Time to 50% mortality at the upper lethal temperatures of:				
	38° C.	35° C.	32° C.	29° C.	27° C.
6° C.	3.5 min. (10)*	10 min. (9)	35 min. (11)	102 min. (8)	662 min. (8)
12°	4.5 " (8)	8 " (13)	46 " (14)	125 " (8)	800 " (7)
15°	7 " (8)	12 " (10)	49 " (10)	172 " (10)	810 " (8)

* The number of specimens used in each experiment are in parentheses.

Table II. Survival time increase for each degree drop in the lethal temperature for Plethodon dunni

Acclim. Temp.	Increase in survival time (min.) /° C. drop in lethal temp.			
	38° to 35° C.	35° to 32° C.	32 to 29° C.	29° to 27° C.
6° C.	2.16	8.33	22.3	280.0
12°	1.16	12.66	26.3	337.5
15°	1.66	12.30	41.0	319.0

I. Table II gives the increase in the survival time in minutes for each degree centigrade drop in the lethal temperature from 38° to 27° . An example of the calculations will clarify the preceeding statement. It is seen from Table I that for 15° acclimation temperature the survival time at a lethal temperature of 29° is 172 minutes, and at a lethal temperature of 27° it is 810 minutes. For a two-degree drop in the lethal temperature (from 29° to 27°) there is an increased survival time of 638 minutes. Thus, P. dunni exhibits a gain of 319 minutes per degree centigrade drop in lethal temperature from 29° to 27° , which is the figure listed in the bottom of the fifth column from the right in Table II. All the figures in Table II have been calculated in this manner. Attention is called to the fact that the greatest relative increase in this species' ability to survive the lethal temperatures tested occurs from 29° to 27° . This phenomenon may be seen more clearly if the figures for 15° , for example, are compared in Table II. The increase in survival time from the 38° to 35° interval to the 35° to 32° interval is nearly twelvefold, and between the interval 35° to 32° and 32° to 29° it is about three and one-thirdfold, whereas between the intervals of 32° to 29° and 29° to 27° the increase of survival time is nearly eightfold.

Plethodon vehiculum

The results of the experiments on the upper lethal temperatures of Plethodon vehiculum as a function of acclimation are given in Table III. The data from Table III are graphically summarized in Figure 3. Figure 3 shows that the 6°-acclimated groups exhibit a change in the slope of the resistance time-curve at a lethal temperature of 32°. The curve representing the 12°-acclimated groups in Figure 3 also changes its angle of slope at the lethal temperature of 32°, but this curve has another change of its slope at the 29° lethal level. In contrast to the 6° and 12°-acclimated groups, those groups acclimated to 15°, as shown in the curve of Figure 3, have an inflection at each lethal level. This is somewhat perplexing, since the curves for 6° and 12° groups show an almost straight-line progression from the higher to the lower lethal temperatures. If a curve were to be fitted by eye to the data for the 15°-acclimated groups, it would be possible to have a continuously changing slope (i.e., a curving line) from the highest to the lowest lethal temperature. This does not seem warranted, however, since Figure 3 also shows that the three acclimation groups did not stratify, one above the other, in the order of survival time at the lethal temperatures used for 6°, 12°, and 15° acclimation. For example, at the lethal temperatures of 38°, 35°, 32°

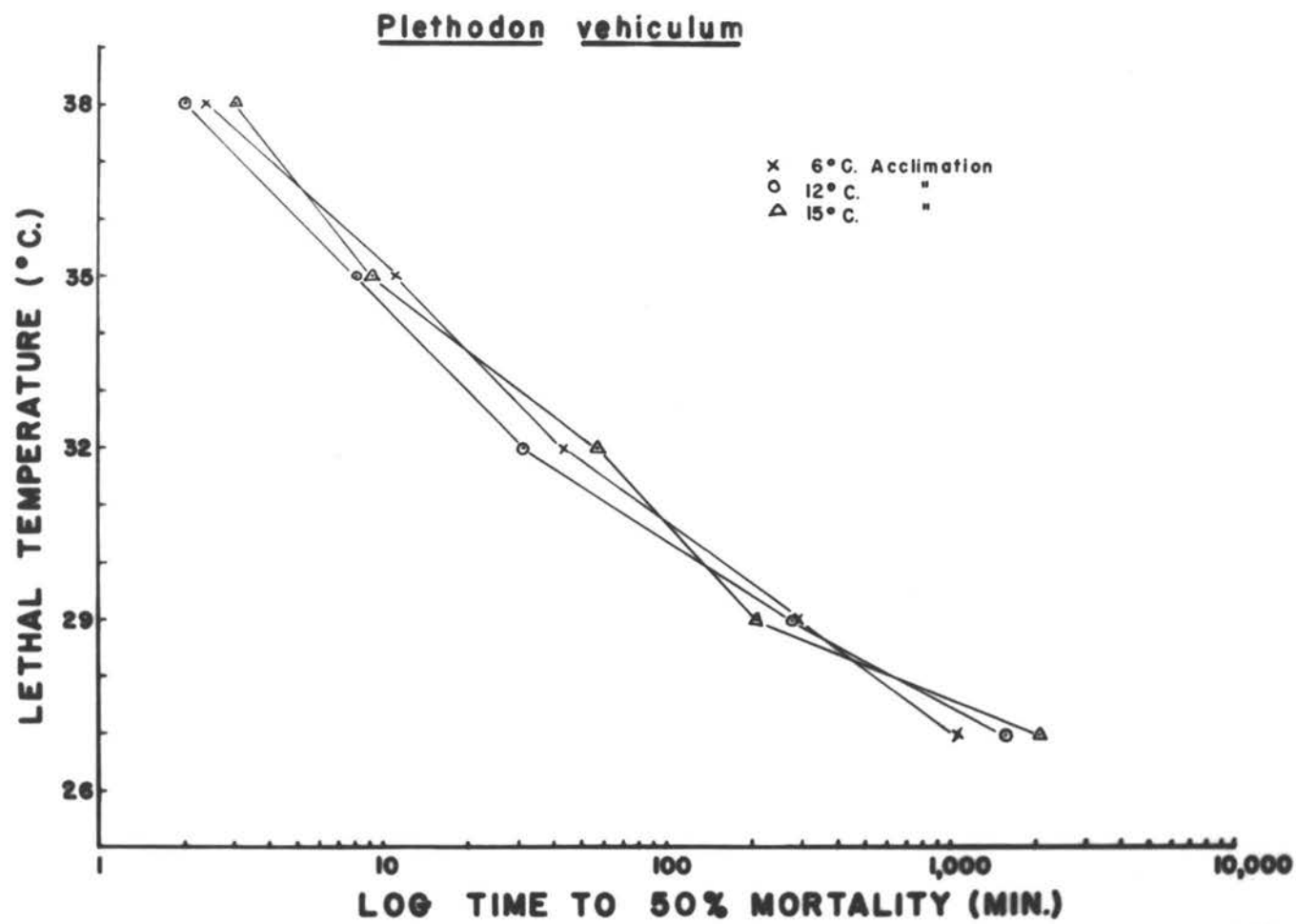


Figure 3

Table III. Data summary of acclimation - lethal temperature experiments on Plethodon vehiculum

Acclim. Temp.	Time to 50% mortality at the upper lethal temperature of:				
	38° C.	35° C.	32° C.	29° C.	27° C.
6° C.	2.25 min. (7)*	11 min. (10)	12 min. (12)	290 min. (10)	1056 min. (8)
12°	2 " (14)	8 " (6)	31 " (8)	280 " (8)	1570 " (8)
15°	3 " (8)	9 " (8)	56 " (10)	205 " (10)	2085 " (8)

*The number of specimens used in each experiment are in parentheses.

Table IV. Survival time increase for each degree drop in the lethal temperature for Plethodon vehiculum

Acclim. Temp.	Increase in survival time (min.) / ° C. drop in lethal temp.			
	38° to 35° C.	35° to 32° C.	32° to 29° C.	29° to 27° C.
6° C.	2.9	0.3	92.7	383.0
12°	2.0	7.7	83.0	645.0
15°	2.0	15.7	49.7	940.0

and 29°, the 6°-acclimated groups survived longer than the 12°-acclimated groups. Further, at the lethal temperatures of 35° and 29°, the 6°-acclimated groups survived longer than the 15°-acclimated groups.

The data of Table III have been reworked in a different manner and presented in Table IV in the same manner as that described for Plethodon dunni on page 25 of this thesis. Table IV illustrates the rapid gain in tolerance between the lethal temperature intervals of 32° to 29° and 29° to 27°. Between these two intervals the increase in survival time is nearly fourfold, eightfold, and nineteenfold for the 6°-, 12°-, and 15°-acclimated groups respectively. Comparison of Table IV with Table II (calculated from P. dunni data) on page 24 shows that P. vehiculum has a higher tolerance to the lethal temperatures between 32° and 27°.

Aneides ferreus

The results of the experiments on the upper lethal temperatures of Aneides ferreus, as a function of acclimation, are given in Table V. The data of Table V are graphically summarized in Figure 4. It will be noticed in the data summary of Table V and in Figure 4 that the lethal temperatures of 29° and 27° were not determined, since the incipient lethal temperatures for the three

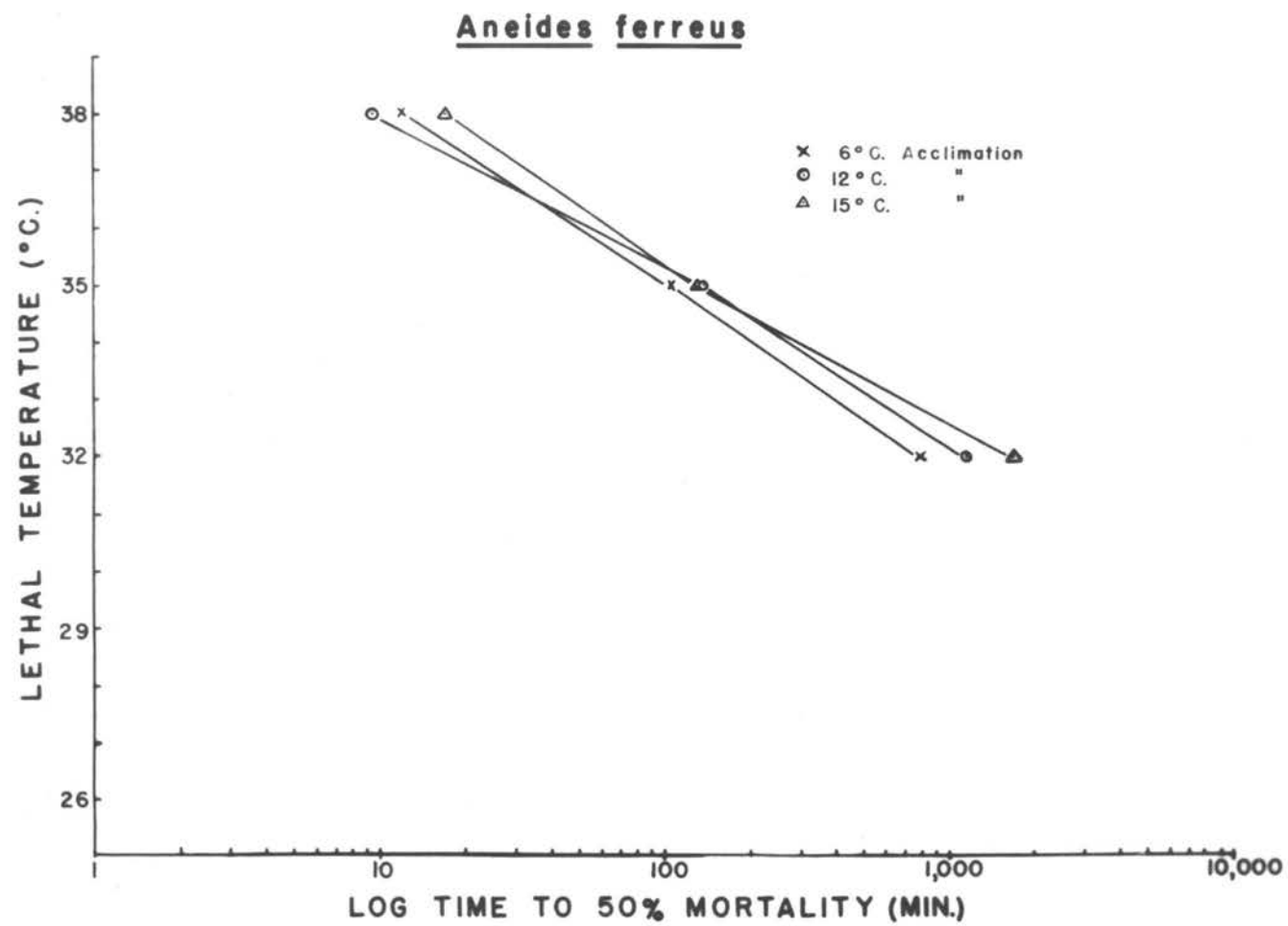


Figure 4

Table V. Data summary of acclimation - lethal temperature experiments on Aneides ferreus

Acclim. Temp.	Time to 50% mortality at the upper lethal temperature of:				
	38° C.	35° C.	32° C.	29° C.	27° C.
6° C.	12 min. (8)*	106 min. (8)	805 min. (8)		
12°	9.5 " (8)	136 " (12)	1150 " (8)		
15°	17 " (8)	130 " (10)	1680 " (10)		

* The number of specimens used in each experiment are in parentheses.

Table VI. Survival time increase for each degree drop in the lethal temperature for Aneides ferreus

Acclim. Temp.	Increase in survival time(min.) /°C. drop in lethal temp.			
	38° to 35°C.	35° to 32°C.	32° to 29°C.	29° to 27° C.
6° C.	31.3	233.0		
12°	42.2	338.0		
15°	37.7	483.0		

acclimation temperatures could be determined at a lethal temperature of 32° (incipient lethal temperature defined on page 15). Comparing Figure 4 with Figures 2 and 3, it is seen that Aneides ferreus has an increased overall survival time to each of the lethal temperatures tested. Furthermore, the gain in survival time for A. ferreus between the intervals of 38° to 35° and 35° to 32° is greater when comparing Table VI with those same intervals of P. dunni (Table II, page 24) or P. vehiculum (Table IV, page 28).

As noted for P. dunni and P. vehiculum, the survival time-curves for A. ferreus in Figure 4 do not show the sequential stratification expected for acclimation. It is seen that the 6°-acclimated group survived longer at a lethal temperature of 38° than did the 12°-acclimated group. Also at a lethal temperature of 35°, the 12°-acclimated group survived slightly longer than the 15°-acclimated group (136 minutes and 130 minutes respectively).

Interspecific Comparisons

For the purpose of comparing the differences between P. dunni, P. vehiculum, and A. ferreus, Figures 5, 6, and 7 were prepared from the data in Tables I, III, and V. Figure 5 is a comparison of the 6°-acclimated groups; Figure 6 is a comparison of the 12°-acclimated groups; and

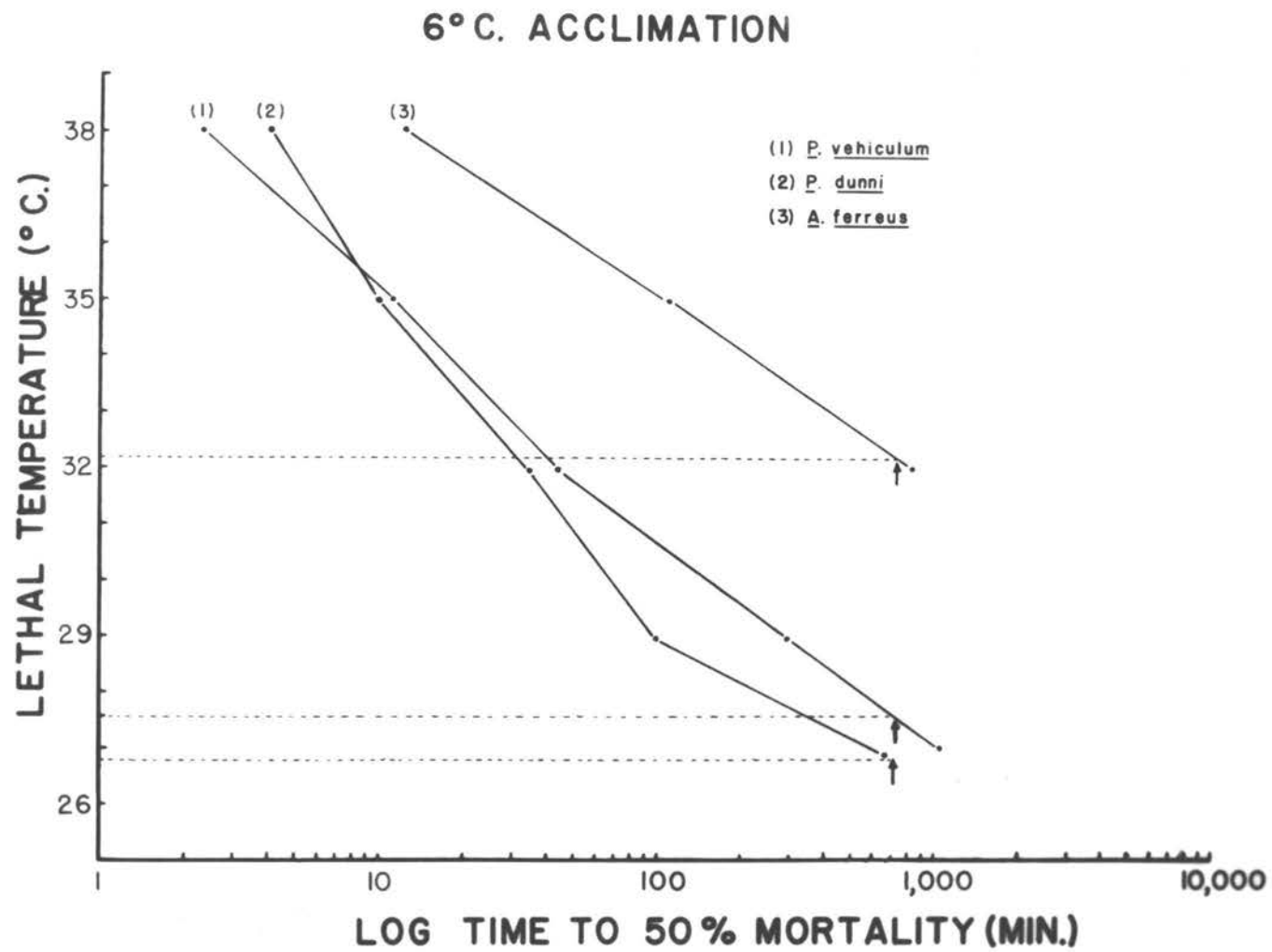


Figure 5

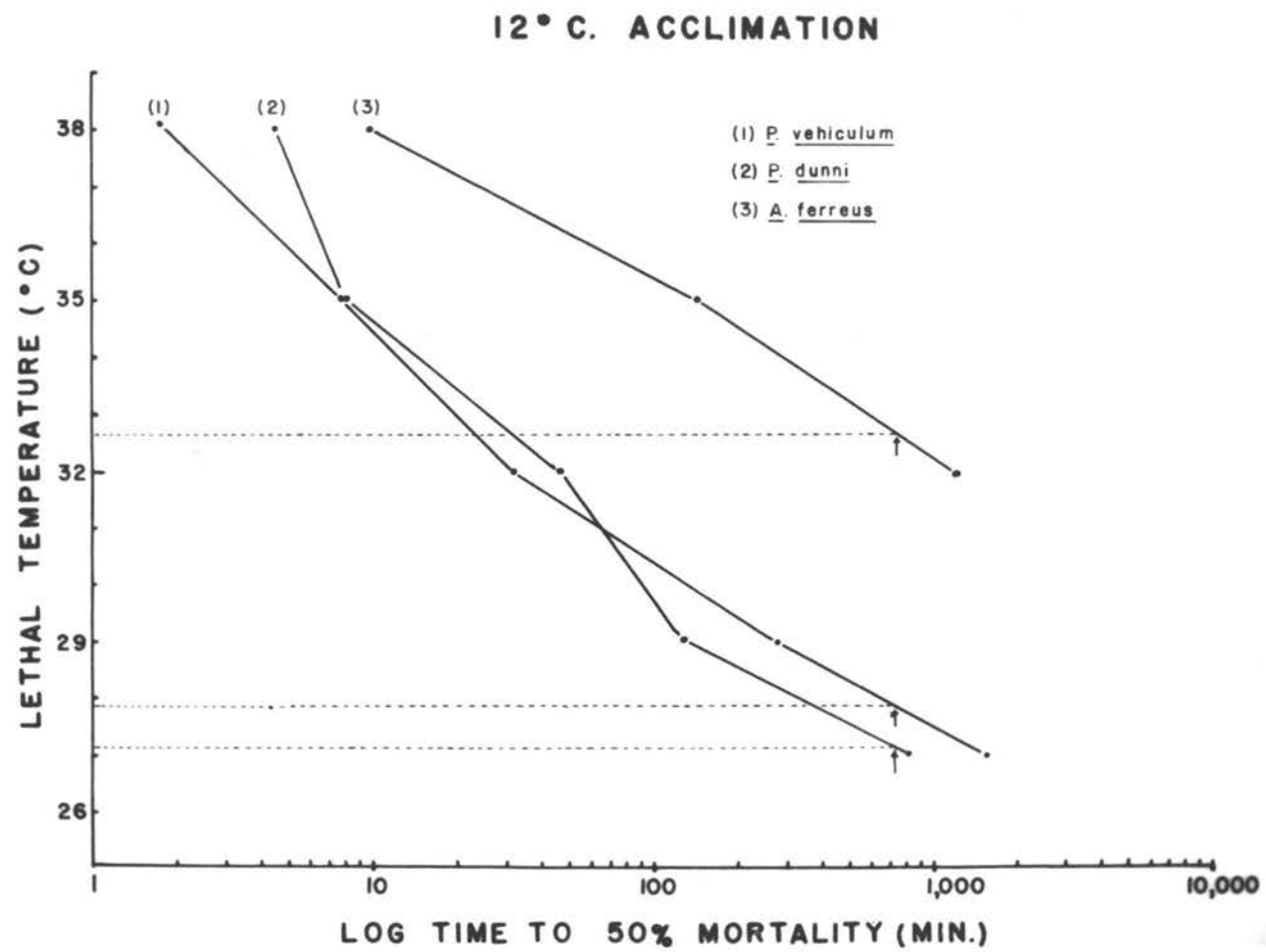


Figure 6

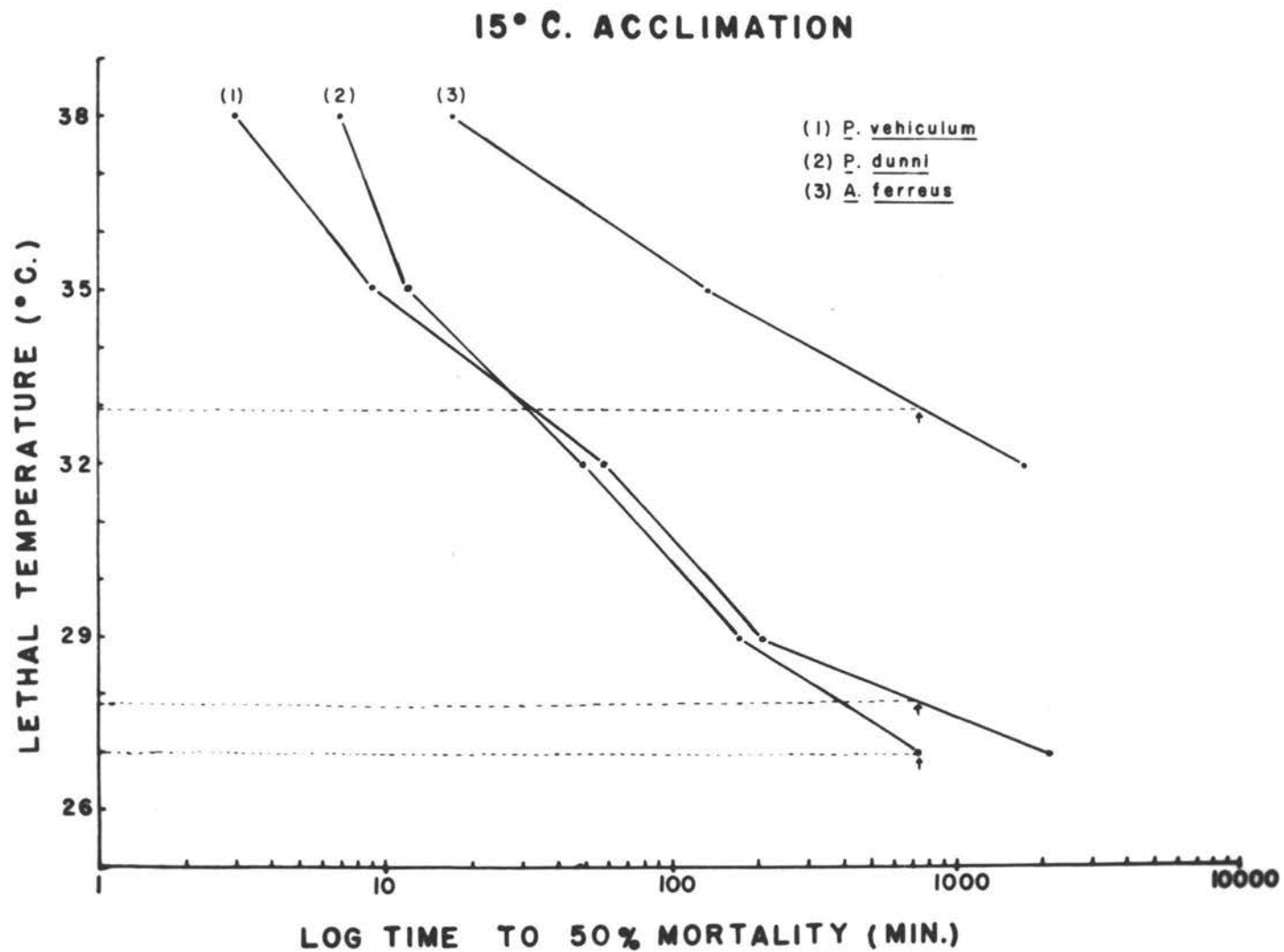


Figure 7

Figure 7 is a comparison of the 15°-acclimated groups. The incipient lethal temperatures for each species are shown in Figures 5, 6, and 7 by an arrow indicating the point of 12 hours, or 720 minutes, on the survival time-curves, and a dotted line carries from this point to the lethal temperature-axis of these Figures, indicating the incipient lethal temperature for the species and acclimation group. The incipient lethal temperature is that temperature at which 50 percent of the sample survives for 12 hours or longer.

The most outstanding phenomenon in Figures 5, 6, and 7 is that A. ferreus possesses the ability to withstand higher lethal temperatures than P. dunni or P. vehiculum. Comparing the curve for P. dunni with that for P. vehiculum in Figures 5, 6, and 7, it is seen that P. dunni has a longer survival time to a lethal temperature of 38° than P. vehiculum, but at a lethal temperature of 27°, P. dunni has less survival time than P. vehiculum.

For each succeeding acclimation level from 6° to 15°, A. ferreus had increased incipient lethal temperatures as follows: 32.2° for 6°, 32.6° for 12°, and 33.0° for 15° acclimation. The survival time of P. dunni at a lethal temperature of 27° for 6° acclimation is not long enough to determine accurately the incipient lethal temperature, but an extension of the curve gives a value of about 26.8°.

From 6° to 12° acclimation, P. dunni exhibited an increase of the incipient lethal temperature from about 26.8° to 27.1°. However, there was no change from the 12° to the 15° acclimation groups since the incipient lethal temperatures were 27.1° for both groups. P. vehiculum showed no increase of incipient lethal temperatures from the 12° to the 15° acclimation levels. Both acclimation groups had incipient lethal temperatures of 27.9°. At an acclimation level of 6°, however, P. vehiculum had an incipient lethal temperature of 27.6°. Thus, P. dunni and P. vehiculum had an increase of 0.3° in their incipient lethal temperatures between 6° and 12° acclimation, and A. ferreus had an increase of 0.4° in the incipient lethal temperature from 6° to 12° and from 12° to 15° acclimation levels.

DISCUSSION

Several authors list extensive tables on low and high lethal temperatures (24, p. 344-345) (14, p. 482-483) (26, p. 434) for various species of animals, among which are some examples of amphibians. These tables are very limited, however, since for most of the lethal temperatures cited, the conditions of the testing procedure and the prior thermal history are not stated or known, and these must be known before these values are to have any basic comparative value. Comparing the upper incipient lethal temperatures of P. dunni, P. vehiculum, and A. ferreus with other species of amphibians is difficult, since other studies performed to date have either neglected to state the thermal history of species in question or have used other criteria not comparable to that used in this study to determine the lethal temperature (e.g., critical thermal maximum).

It is not known if the criterion of the critical thermal maximum (CTM) may be compared, on a relative scale, with incipient lethal temperatures. This aspect was not experimented on in this study, nor does any such study exist in the literature. Zweifel's data (see page 7) indicated acclimation for two species of East Coast plethodontid salamanders, Desmognathus quadrimaculatus and D. f.

fuscus, comparing 5° and 15° acclimation samples and using the CTM as his criterion. Zweifel's technique was to heat the salamanders at a steady rate from the temperature at which they were acclimated to a point of locomotor paralysis, which took from ten to 15 minutes. The temperature of the salamanders at the first sign of paralysis was considered to be the critical thermal maximum.

Dumas (9, p. 488) determined the time-temperature relationships for P. dunni and P. vehiculum by subjecting the specimens to various lethal temperatures and recording the time to paralysis. If the 15-minute survival rates are comparable to the critical thermal maximum of Zweifel, then Dumas showed that both P. dunni and P. vehiculum have a critical thermal maximum of about 32°. However, Dumas does not state the previous thermal history of his salamanders, which limits the value of his determinations in this discussion. In this study, the highest upper incipient lethal temperature determined for P. dunni was found to be 27.1°, and for P. vehiculum it was 27.9°. In an indirect manner, it has been shown that the critical thermal maximum and the upper incipient lethal temperature are not the same, at least for P. dunni and P. vehiculum, but it does not prove that relative comparisons of the two types of criteria are invalid.

It was seen that P. dunni and P. vehiculum did not show any increase of the upper incipient lethal temperatures for 12° and 15° acclimation. This is not altogether unknown in the literature, since Fry, Hart, and Walker (12, p. 14) reported that the speckled trout, Salvelinus fontinalis, had an upper incipient lethal temperature of 25.3° for 20°, 24° and 25° acclimation. In another study, Doudoroff (8, p. 230) reported that the greenfish, Girella nigricans, had an upper incipient lethal temperature of 29.0° for 12° acclimation, an upper incipient lethal of 32.3° for 20° acclimation, and an upper incipient lethal of 32.5° for 28° acclimation. Doudoroff's experiments, then, showed an increase of the upper incipient lethal temperature of 3.3° between 12° and 20° acclimation and of only 0.2° between 20° and 28° acclimation. At about 20°+ the greenfish was almost at its genotypic limit of the zone of thermal tolerance, and beyond 28° acclimation the species would probably enter its zone of resistance, or the zone in which thermal acclimation is ineffective (8, p. 239). This reasoning applied to Fry, Hart, and Walker's results (vide supra) showed that the genotypic limits of acclimation were at 20° acclimation, since acclimation to 24° and 25° did not increase the upper incipient lethal temperature.

It was pointed out in the results section of this thesis that, for all three species of salamanders tested, the acclimated groups did not always stratify, one above the other, in the order of survival times at the lethal temperatures used for 6°, 12° and 15° acclimation. This variance was not expected, since it was believed at the start of this study that P. dunni, P. vehiculum, and A. ferreus would demonstrate acclimation. This variance may be explained in part, on the variability of the species' resistance to lethal temperatures. To help prove this point, reference to Zweifel's data again (page 7, this thesis) will show variability of response to lethal temperatures. For example, Zweifel reported a CTM range of 29.5° to 30.5° for Desmognathus quadrimaculatus acclimated to 5°, and at 15° acclimation he reported that this species had a range of 31.0° to 32.0°. Another example showing greater variation of the CTM has been reported by Cole (5, p. 97) who found a variation of 40.3° to 49.0° with a mean value of 43.74° for the fence lizard, Sceloporus undulatus. The technique of determining the CTM is to heat the animal at a steady rate from the temperature at which it was acclimated to the point of paralysis in a short interval of time (15 minutes in Zweifel's study and 130 minutes in Cole's). The body temperature of the animal at the point of paralysis was

the CTM. Thus, in the CTM studies, the time to death was held relatively constant but the temperature was changing (i.e., increasing). In the present investigation, the lethal temperature was held constant and the time to mortality was changing. If a 1° variation in the CTM reported by Zweifel (vide supra), with the time to death held constant, were to be compared with the technique of this study, where the temperature was held constant, then a variation in the time to CTM would be expected.

Another factor which would possibly account for the nonstratification of the survival times would be the method of recording the LD₅₀ (50 percent of the sample surviving some entity). In this study, the time at which one-half the sample expired was used as the LD₅₀ time for a given acclimation-lethal temperature sample. This time does not always correspond to the arithmetic mean of the 50 percent survival of the sample. For example, in one experiment, using Aneides ferreus acclimated to 6° and subjected to a lethal temperature of 35° , survival times to expiration were 81, 81, 106, 106, 127, 143, 143 and 162 minutes. The figure used to plot the curves in this study was 106 minutes, or that point where 50 percent of the specimens had expired, and not the arithmetic mean of the range of survival times which would be 116.1 minutes. In this particular instance, it is seen that if 116 minutes were used

instead of 106 minutes, the curve for A. ferreus at 6° acclimation in Figure 4 (page 30) would be changed very little. Unfortunately, only a few of the samples were recorded in such a way as to note the survival time for each specimen of a sample. Hence, it is not known definitely if the method used for recording the LD₅₀ in this study contributes to the variability of the curves or not.

Some of the foregoing discussion has been devoted to the possible explanations of the nonstratification of the lethal temperature-50 percent mortality time curves for the different levels of acclimation temperature for the three species used in this investigation. This was done from the view that these species could or would demonstrate acclimation to temperature. If the opposite view were maintained, then the variability of the curves would have to be explained on the basis of the variability of the response to lethal temperatures of the various samples. The position that Plethodon dunni and Plethodon vehiculum do not acclimate to temperature, at least to the thermal levels tested, appears to be the correct one. It will be remembered that P. dunni gained only 0.3° in its upper incipient lethal temperature for the same acclimation interval. However, both species did not show any increase in their upper incipient lethals beyond 12° acclimation.

If the criterion of the upper incipient lethal temperature has any real meaning, which it has in fishes, then the results indicate that P. dunni and P. vehiculum acclimate very little or not at all. It is possible, however, that acclimation to temperature could be demonstrated if a metabolic rate function (e.g., oxygen consumption) were used as the criterion.

The results for A. ferreus indicate acclimation to temperature in contrast to P. dunni and P. vehiculum. A. ferreus showed 0.8° increase in its upper incipient lethal temperature from an acclimation temperature of 6° to 15° . An increase of 0.8° does not compare to an increase of 3.3° in the upper incipient lethal temperature reported for the greenfish, Girella nigricans, by Doudoroff (8, p. 23) between the acclimation interval of 12° to 20° , but it does compare favorably with the data of Fry et al. (12, p. 20) who reported acclimation in the speckled trout, Salvelinus fontinalis, which showed an increase of 1° in the acclimation interval of 6° to 15° .

Plethodon dunni, Plethodon vehiculum and Aneides ferreus are largely sympatric in Oregon, and they often occupy the same habitat. It might be expected that these species would have the same or similar thermal tolerances, but this study has shown that the order of increasing thermal tolerance for these species would be P. dunni,

P. vehiculum, and A. ferreus (pages 33 to 35). Of these three species, A. ferreus shows a very marked increase of thermal tolerance. This fact might be correlated with the distribution of A. ferreus, since this species' range is much farther south than either of the other two species. Further, A. ferreus is not restricted to the "Rocky Outcrop-Talus Slope Ecosystem" but is also found in rotting Douglas fir stumps and logs in clearings where the timber has been logged and second growth has started. In this habitat, the niche temperature could get rather high. It might be speculated that the higher resistance to upper temperatures found in A. ferreus could be the factor that would allow the species to be rather adaptable in habitat selection.

On the other hand, P. dunni and P. vehiculum had lower incipient lethal temperatures and did not show any significant degree of acclimation. Both of these species are rather restricted in their habitat. They are both found in rock outcrops and talus slopes that often have water seepages (9, p. 485). The niche temperatures recorded by Dumas (9, p. 487) in this habitat vary from 4° to 19°, but the majority of the measurements fall between 7° and 14°. It might tentatively be concluded from this that their habitat does not undergo much of a temperature change, but is a rather stable environment with respect to

temperature, or else these species avoid higher temperatures by migrating in the substratum. The lack of acclimation to temperature might be correlated, as one of the factors, with the restriction of P. dunni and P. vehiculum to the "Rocky Outcrop-Talus Slope Ecosystem."

SUMMARY AND CONCLUSIONS

Temperature, as one of the physical factors of the environment, is often important in controlling the actual distribution and abundance of species in nature, either by itself or in conjunction with other environmental factors. The existence of thermal adjustment and changes in metabolism, as a result of a change in the thermal environment, is a well established phenomenon in nature. Acclimation, or the short-term adjustment of the species' systematic organization in direct response to changes in temperature, has been little studied in salamanders. This study is concerned with the demonstration of the presence and amount or the absence of acclimation to temperature in three species of plethodontid salamanders: Plethodon dunni, Plethodon vehiculum, and Aneides ferreus.

The salamanders were acclimated to 6°, 12°, and 15° C. levels for a period of seven days without feeding. They were exposed to the upper lethal temperatures of 38°, 35°, 32°, 29°, and 27° C. in the order given until a 50 percent survival time of twelve hours was obtained to determine the upper incipient lethal temperature for each acclimation level.

The order of thermal tolerance was: P. vehiculum, P. dunni, and A. ferreus. P. dunni had the upper incipient

lethal temperatures of 26.8°, 27.1° and 27.1° C. for the acclimation temperatures 6°, 12°, and 15° C. respectively. P. vehiculum had the upper incipient lethal temperatures of 27.6°, 27.9° and 27.9° C. for the acclimation temperatures 6°, 12° and 15° C. respectively. A. ferreus had the upper incipient lethal temperatures of 32.2°, 32.6° and 33.0° C. for the acclimation levels of 6°, 12° and 15° C. respectively.

From the very small increase in the upper incipient lethal temperatures, coupled with the variation of the survival times for different lethal temperature-acclimation temperature experiments, for P. dunni and P. vehiculum, it was concluded that they show little, if any, thermal acclimation. The increase in the upper incipient lethal temperature for A. ferreus was concluded to be enough to demonstrate thermal acclimation.

The ability to acclimate to temperature was used to correlate tentatively the distribution and habitat selection of A. ferreus. A. ferreus extends farther south and occupies the "Rocky Outcrop-Talus Slope Ecosystem" and the rotting Douglas fir log habitats.

The lack of the ability to acclimate to temperature was used to correlate tentatively the habitat restriction of P. dunni and P. vehiculum to the relatively thermally stable "Rocky Outcrop-Talus Slope Ecosystem" habitat.

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