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ROLE OF TEMPERATURE AND WATER IN THE ECOLOGY OF LUNGLESS SALAMANDERS¹

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ABSTRACT

Water economy and thermal relations of plethodontid salamanders were studied in the laboratory and in the field. Laboratory measurements included behavioral responses in temperature and relative humidity gradients, rates of dehydration and rehydration at various relative humidities and soil-moisture levels, and determination of critical thermal maxima by rapid controlled heating to a definite endpoint. Salamanders were acclimated to a combination of three temperatures and two photoperiods: 5°C, 16 hr of light alternating with 8 hr of darkness (LD 16:8); 5°C, (LD 8:16); 15°C (LD 16:8); 15°C (LD 8:16); 25°C (LD 16:8); and 25°C (LD 8:16). Three series of experiments were conducted on 20 populations representing 14 species.

Critical thermal maximum increased with an increase in acclimation temperature indicating that the salamander's heat resistance was readily altered by its previous thermal history. Salamanders selected a definite range of temperatures and did not merely avoid extremes in the thermal gradient. Thermal preferenda were relatively stable for each species and were not significantly affected by either acclimation temperature or photoperiod. Rate of dehydration was dependent upon body size, drying power of the air, and ambient temperature. Interspecific differences in dehydration rates appeared to be related in part to differences in size. Species composed of small individuals lost weight faster than species made up of large individuals. As the vapor pressure deficit increased the dehydration rate increased. Dehydration was more rapid at higher temperatures. Rehydration rate increased as the percentage of weight loss due to dehydration increased and was more rapid at higher temperatures. Salamanders absorbed water from soil when soil-moisture tension was as high as 2.8 atm at 25°C. All species absorbed water from unsaturated soil at similar rates that were dependent upon soil-moisture content. Salamanders in the humidity gradient responded positively to differences in the moisture content of the air, and all but one species were more than 70% successful in selecting the highest relative humidity available in the gradient. Interspecific differences were apparent, but were not always correlated with habitat preferences.

Plethodon glutinosus did not show any specific adaptation in its thermal responses or water relations that would account for its widespread distribution in the eastern United States. *Plethodon ouachitae* and *P. caddoensis* apparently have survived in the Ouachita Mountains due to favorable microhabitats and their ability to burrow deep beneath talus-covered slopes during hot and dry summers. Surface activity of *P. caddoensis* is limited in the summer by hot and dry conditions in its microhabitat.

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INTRODUCTION

Numerous studies have shown that temperature and water are critical factors in the survival of salamanders in terrestrial environments. Hutchison (1961) noted the low tolerance of salamanders to high temperatures, and Brattstrom (1963) reported that, except for the ability to adjust to lethal temperatures by acclimation, their only body-temperature control was through behavioral adjustments. Other investigations have shown that amphibians constantly lose water by evaporation even in saturated environments (Adolph 1932, Thorson 1955). Spight (1968) reported that terrestrial salamanders and free water surfaces of equal area lose water through evaporation at similar rates, and Littleford, Keller, and Phillips (1947) demonstrated that body water losses exceeding 18–26% were lethal in some plethodontid salamanders. These earlier studies provide a background for additional research into the effects of temperature and water on the adaptation of salamanders to terrestrial habitats. The purpose of this investigation was to determine the role of these factors in the ecology, habitat selection, niche segregation, and distribution of a number of closely related species of salamanders of the family Plethodontidae.

Salamanders of the genus *Plethodon* are terrestrial, completing their entire life cycle without entering water. The *yonahlossee* group of the eastern large plethodonts includes *P. yonahlossee* Dunn and *P. longicrus* Adler and Dennis of the southern Appalachian Mountains and *P. ouachitae* Dunn and Heinze and *P. caddoensis* Pope and Pope of the Ouachita Mountains of Arkansas and Oklahoma (Adler and Dennis 1962, Highton 1962b). These closely related species are widely separated geographically and subjected to different climatic regimes. The *glutinosus* group includes *P. glutinosus* (Green) and *P. jordani* Blatchley. The former is widely distributed throughout the eastern United States and is found within the ranges of all the species of the *yonahlossee* group. The latter is found in the southern Appalachians and is sympatric with *P. yonahlossee* and *P. longicrus*. These species were studied to determine the effects of water and temperature on the ecology and distribution of closely related salamanders.

In both North Carolina and Arkansas several other species of the family Plethodontidae occur at the same sites as those species already mentioned. These species were also studied to determine the effects of temperature and water on habitat selection and niche segregation among sympatric plethodontid salamanders.

METHODS

General

Most salamanders were collected from March to October in 1968 and 1969, brought back to the lab-

oratory, and stored at 5°C in a darkened refrigerator. *Plethodon ouachitae* was collected on Rich Mountain, Polk County, Arkansas, and *P. caddoensis* was collected on a north-facing slope in Camp Albert Pike Park, Montgomery County, Arkansas. *Plethodon glutinosus* (hereafter referred to as *P. glutinosus* (Ouachita)) and *P. cinereus serratus* Grobman were collected on Rich Mountain and in Camp Albert Pike. *Desmognathus fuscus brimleyorum* Stejneger was collected from a stream near Camp Albert Pike (referred to as *D. fuscus* (AP)) and on Rich Mountain (referred to as *D. fuscus* (RM)). *Plethodon glutinosus* (referred to as *P. glutinosus* (Ozark)) and *Eurycea lucifuga* Rafinesque were collected at the following localities in the Arkansas Ozarks: Logan Springs cave, 10 miles west of Tontitown, Benton County; Delap caves, 4 miles west of Prairie Grove, Washington County; Denny cave, 3 miles northeast of Alabam, Madison County; War Eagle cave, 18 miles east of Rogers, Benton County; and Granny Dean cave, Black Oak Community, Washington County. *Plethodon dorsalis angusticlavius* Grobman (referred to as *P. dorsalis*) was collected at Denny cave, War Eagle cave, and Granny Dean cave. *Eurycea longicauda melanopleura* (Cope) (referred to as *E. longicauda*) was collected at Logan Springs cave, War Eagle cave, Granny Dean cave, Delap caves, and the Ozark Trout Farm, Johnson, Washington County. *Eurycea multiplicata* (Cope) was collected at the Ozark Trout Farm. *Plethodon yonahlossee*, *P. jordani*, *P. glutinosus* (referred to as *P. glutinosus* (NC)), *P. cinereus cinereus* (Green), *D. ochrophaeus carolinensis* Dunn (referred to as *D. ochrophaeus* (NC)), and *D. monticola monticola* Dunn (referred to as *D. monticola*) were collected along Old Yonahlossee Road, Grandfather Mountain, Avery County, North Carolina. *Plethodon jordani* (referred to as *P. jordani* (SM)) and *D. o. carolinensis* (referred to as *D. ochrophaeus* (SM)) were also collected near Indian Gap, Great Smoky Mountains National Park, Sevier County, Tennessee. *Plethodon longicrus* was collected at Bat Caves, Rutherford County, North Carolina.

Experimental animals were acclimated to three experimental temperatures in combination with two photoperiods for a minimum of 1 week prior to testing. Acclimation states were: 5°C, 16 hr of light alternating with 8 hr of darkness (LD 16:8); 5°C (LD 8:16); 15°C (LD 16:8); 15°C (LD 8:16); 25°C (LD 16:8); and 25°C (LD 8:16). All animals were acclimated in constant temperature ($\pm 1^\circ\text{C}$) chambers. Illumination was provided by two 40-w fluorescent lamps controlled by automatic switching devices. Photoperiods were centered at 12:00 noon CST or CDT. Salamanders were kept on moist paper towels in 8-inch culture dishes during storage and acclimation. They were tested within 6 months after

capture. At 15° and 25°C they were fed mealworms, vestigial wing fruit flies (*Drosophila melanogaster*), and small worms. At 5°C they were force-fed pieces of chopped beef liver and strained beef baby food. Feeding was stopped at least 2 days prior to testing. Sick or injured animals were not tested. Individual animals were identified by toe clipping and tested at only one acclimation state. Animals were undisturbed during acclimation except when bowls were changed and during force-feeding. They were subjected to short disturbances during the experimental periods when animals were removed or returned in conjunction with testing.

Three series of experiments were conducted. In the first, groups of *P. ouachitae*, *P. glutinosus* (Ozark), *P. jordani*, and *P. glutinosus* (NC) were brought to all six acclimation states and tested. Two replications were completed; about 10 animals from each population were tested at each acclimation condition. The animals in the first series were designated as group I. In the second series, groups of from 8 to 14 *P. yonahlossee*, *P. caddoensis*, and *E. lucifuga* were acclimated to 5°, 15°, and 25°C at a constant photoperiod (LD 16:8). These species were group II. In the third series the remainder of the salamanders (group III) was acclimated to 15°C (LD 16:8) and tested. In all cases, test temperatures were the same as acclimation temperatures.

Tests included the response to temperature and relative humidity gradients, rates of dehydration and rehydration at various relative humidities and soil-moisture levels, and determination of critical thermal maxima (CTM) by rapid controlled heating to a definite endpoint.

Statistical

A different experimental design was used for each test group. A factorial split-plot design was used with group I, a randomized block design with group II, and a completely random design with group III. A split-plot analysis of variance (ANOVA) was performed on experimental data for group I. A two-way ANOVA was used for experiments with group II and a one-way ANOVA for experiments with group III. Duncan's new multiple range test was used to align mean values of data for group III according to their significance levels. Because each group was subjected to a different experimental design and statistical analysis, the results for each group are presented separately. The analysis of covariance (ANCOVA) was performed on the third set of dehydration experiments (dehydration at high relative humidities) and on the tests of the rate of water exchange with soil. Analysis of covariance was also used to determine the effects of various independent factors on the results of the tests used in this study. These factors included weight and time of day in

the CTM test; time of day in the gradient tests; weight in the dehydration tests; and weight, percentage weight loss, and time period in the rehydration tests.

The model equation for the ANCOVA with one covariate is

$$Y = \mu + \alpha_1 + \beta X + \epsilon,$$

where μ is the population mean, α_1 is the deviation of the mean of the regression line from the population mean, β is the slope of the regression line, X is the independent variable, and ϵ is a random variable. In the case of two covariates, such as weight and time of day in the CTM tests, the equation is

$$Y = \mu + \alpha_1 + \beta_1 X_1 + \beta_2 X_2 + \epsilon,$$

where X_1 = weight and X_2 = time of day. A *t*-test was performed on the estimated β to determine if the covariate had a significant effect on the Y -value. Calculated values were considered significant at the 5% level of probability ($P \leq 0.05$) and highly significant at the 1% level ($P \leq 0.01$).

CRITICAL THERMAL MAXIMUM

The critical thermal maximum (CTM) of a species is a measure of its thermal tolerance and can be used to determine its ability to acclimate to changes in temperature and photoperiod. The initial definition of the CTM by Cowles and Bogert (1944) was modified by Lowe and Vance (1955), who stated that the CTM of a population "... is the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death." Thus the CTM is the point where the animal is unable to escape from conditions that, if continued, will quickly lead to death. If left at this temperature for a short time it enters a coma, undergoes heat rigor, and dies. The CTM should be considered an ecological value since all animals can fully recover from an exposure to CTM, if removed to a lower temperature immediately after exposure. The CTM is distinct from the lethal temperature, the point at which the animal is physiologically dead and cannot recover when placed in a cool environment (see discussion by Fry (1967) for a comparison of CTM and lethal temperature). In this study critical thermal maxima (CTM) were determined according to the definition of Lowe and Vance (1955) as modified by Hutchison (1961). Onset of spasms was used as the endpoint.

Methods

Critical thermal maxima were determined between 0740 and 2300 CST in a lighted laboratory according to the method described by Hutchison (1961). Animals were placed in continually aerated water at

their acclimation temperature and heated at a rate of $1.0^\circ \pm 0.2^\circ\text{C min}^{-1}$. A heating rate of $0.6^\circ \pm 0.2^\circ\text{C min}^{-1}$ was used to test salamanders that weighed more than 8.00 g. Water temperatures were measured with a standardized mercury thermometer graduated to 0.1°C . Deep body temperatures were not recorded since the heating rates used prevented any measurable lag between the temperature of the water bath and that of the salamander. This procedure has been used for salamanders (Zweifel 1957, Hutchison 1961, Whitford 1968) and small frogs (Heatwole, Mercado, and Ortiz 1965, Dunlap 1968).

The onset of spasms was used as the endpoint for the CTM of all individuals tested. This endpoint is not ideal, since the spasms often consist of only a slight twitching of the legs or head and sometimes are signified only by a general loss of locomotory ability and stiffness of the body, but it is consistent. Brattstrom (1960, 1963, 1968) and Brattstrom and Lawrence (1962) have used the loss of righting response as the endpoint for the CTM. Brattstrom (1968) suggested that a new term "onset of spasms" (OS) be applied to that point at which spasms begin and that the loss of righting response be used as the criterion for the CTM. However, the loss of righting response in salamanders is often transitory, does not prevent coordinated locomotion, and many times does not even appear. These findings and the work on CTM of turtles by Hutchison, Vinegar, and Kosh (1966), and on frogs by Heatwole et al. (1965) and more recently by Mahoney and Hutchison (1969), make the definition of this new term (OS) unwarranted. Despite the comments of Brattstrom (1970) I suggest that the onset of spasms be used as the endpoint for CTM. Continued discussion of OS as a term separate from CTM will only cause confusion and increase the ambiguity of results.

Results

The behavior of salamanders in the test apparatus was essentially the same as that described by Hutchison (1961). The endpoint was often very pronounced as evidenced by violent spasms, but sometimes was indistinct, characterized by slight jerking of the legs, spasmotic opening and closing of the mouth, or slight twitches of the entire body followed by complete paralysis. The CTM was not significantly affected by the weight of the animal or the time of day at which the test was conducted. However, an increase in acclimation temperature resulted in a highly significant increase ($P \leq 0.01$) in the CTM of populations in groups I and II. The means for all four populations in group I acclimated to 15°C (Fig. 1) were identical (34.3°C). Species differences (Fig. 2) were highly significant ($P \leq 0.01$) in the group II experiments (randomized block). *Eurycea lucifuga*

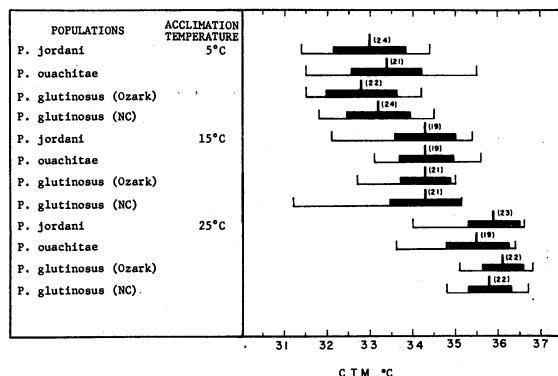


FIG. 1. Critical thermal maximum (CTM) of *P. jordani*, *P. ouachitae*, *P. glutinosus* (Ozark), and *P. glutinosus* (NC) acclimated to 5° , 15° , and 25°C (two light regimes at each temperature are pooled into one sample). Means are represented by long vertical lines. Horizontal lines bounded by short vertical lines represent the ranges. Black rectangles represent ± 1 standard deviation (SD). Number of observations in parentheses.

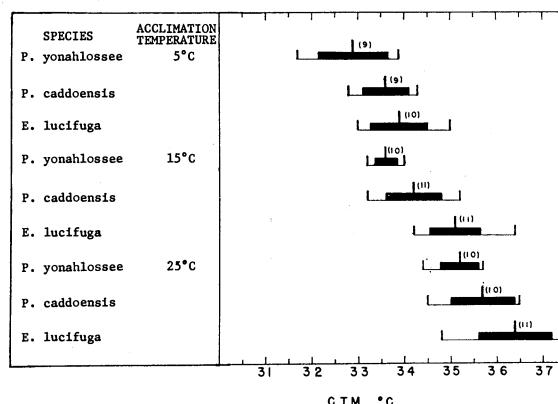


FIG. 2. Critical thermal maximum (CTM) of *P. yonahlossee*, *P. caddoensis*, and *E. lucifuga* acclimated to 5° , 15° , and 25°C (LD 16:8). See Fig. 1 for explanation of symbols.

had the highest CTM at each acclimation temperature, and *P. yonahlossee* had the lowest.

The differences in the CTM of the 13 populations of group III acclimated to 15°C (LD 16:8) were highly significant ($P \leq 0.01$). These differences were clarified by Duncan's new multiple range test (Table 1) and are presented graphically in Fig. 3. *Eurycea longicauda* had the highest mean CTM (34.8°C), and *P. dorsalis* had the lowest (32.3°C).

Discussion

Acclimation to temperature.—The increase in CTM with an increase in acclimation temperature indicates that the heat resistance of these salamanders is readily altered by their past thermal history. This conclusion is consistent with the results of numerous other studies on salamanders (Zweifel 1957, Hutchison 1961, Brattstrom and Regal 1965, Seale and West 1969) and frogs (Brattstrom and

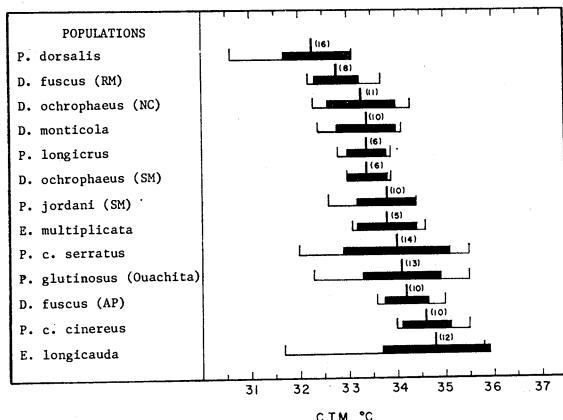


FIG. 3. Critical thermal maximum (CTM) of 13 populations of salamanders acclimated to 15°C (LD 16:8). See Fig. 1 for explanation of symbols.

TABLE 1. Mean critical thermal maximum (CTM) of 13 populations of salamanders acclimated to 15°C (LD 16:8) (group III)

Population	Number of observations	CTM (°C) ^a
<i>P. dorsalis</i>	16	32.3
<i>D. fuscus</i> (RM)	8	32.8
<i>D. ochrophaeus</i> (NC)	11	33.3
<i>D. monticola</i>	10	33.4
<i>P. longicrus</i>	6	33.4
<i>D. ochrophaeus</i> (SM)	6	33.4
<i>P. jordani</i> (SM)	10	33.8
<i>E. multiplicata</i>	5	33.8
<i>P. c. serratus</i>	14	34.0
<i>P. glutinosus</i> (Ouachita)	13	34.1
<i>D. fuscus</i> (AP)	10	34.2
<i>P. c. cinereus</i>	10	34.6
<i>E. longicauda</i>	12	34.8

^aAny two means not encompassed by the same vertical line are significantly different as determined by Duncan's new multiple range test.

Lawrence 1962, Brattstrom 1968, Dunlap 1968, Mahoney and Hutchison 1969). This adaptive response in salamanders results in an increase in their heat tolerance during the summer.

The mechanism by which heat resistance is modified in vertebrate poikilotherms is not understood. Precht, Christopherson, and Hensel (1955) hypothesized that changes in the ratio of bound to free water are closely associated with changes in resistance to temperature extremes. Schmidt-Nielsen and Forster (1954) reported an increase in body weight of *Rana clamitans* (Rafinesque) at 2°–5°C, and Hutchison (1961) noted an increase in weight with a decrease in CTM of newts (*Notophthalmus viridescens* (Rafinesque)) acclimated to 4°C. Changes in acclimation temperature of goldfish (*Carassius auratus* L.) bring about changes in their water and lipid composition (Hoar and Cottle 1952) and alterations in the quantity and composition of their brain plasmalogens (Roots and Johnston 1968).

Ushakov (1964) and Licht (1967) found that differences in the thermostability of tissues, cells, and enzymes parallel interspecific differences in the heat resistance of lizards. Johansen (1967, 1968) reported that the pituitary gland was involved in the heat resistance of goldfish and hypothesized that a prolactin-like hormone played an essential role in resisting high temperature stress. Differences in substrate inhibition between groups of lactate dehydrogenase allotypes in populations of *Rana pipiens* Schreber are related to temperature differences in their ranges (Salthe 1968). These findings suggest that alterations in heat resistance due to temperature acclimation probably involve changes in the structure of enzymes and cellular membranes which give them increased thermostability and allow them to function more efficiently at high ambient temperatures.

Effect of photoperiod.—Changes in heat resistance coincident with altered light regimes have been reported for fish (Hoar 1955, 1956, Hoar and Robertson 1959), frogs (Brattstrom 1968, Mahoney and Hutchison 1969, Dunlap 1969), and turtles (Hutchison and Kosh 1965), and changes in metabolic rate due to photoperiod effects have been reported for salamanders (Whitford and Hutchison 1965a). The lack of any effect of photoperiod on the CTM of salamanders in these experiments may have been due to the low quality or quantity of light in the acclimation chambers or to the use of some unnatural combinations of temperature and photoperiod, such as 25°C with 8 hr light-16 hr dark or 5°C with 16 hr light-8 hr dark.

Species differences.—Some differences in CTM are correlated with differences in the thermal regimes of salamander habitats. *Desmognathus fuscus* from Camp Albert Pike, which were collected from a temporary stream at 960 ft elevation, had a higher CTM than the *D. fuscus* associated with rock glaciers from 1,740–2,100 ft elevation on Rich Mountain (Table 1). *Eurycea lucifuga* from Arkansas have a higher CTM than *E. lucifuga* from Virginia (Hutchison 1961, Sealander and West 1969) and plethodonts from both Arkansas and North Carolina.

Heat resistance was similar among many congeneric species. All plethodonts from the Ouachita Mountains had similar CTM's at 15°C. The CTM's of most plethodonts in groups I and II were similar. Among these salamanders *P. yonahlossee* demonstrated the least adaptation to high temperatures with the lowest CTM at 15° and 25°C. The CTM of *P. longicrus* was similar to that of *P. jordani* from the Great Smoky Mountains despite the fact that these two species encounter quite different climatic conditions. The former was collected at 5,100 ft elevation, and the latter was found at 1,500 ft. The low CTM of *P. dorsalis* indicated that it had less resis-

tance to high temperatures than the other salamanders tested at 15°C. Eastern *Desmognathus* were collected in similar habitats and had similar CTM's.

PREFERRED TEMPERATURE

The thermal preferendum of a species is that narrow range of temperatures which defines its normal activity range. The mean value of this zone of preference is referred to as the preferred temperature (PT). The PT is termed the "preferred body temperature" (PBT) by Bogert (1949) and corresponds to the "ecritic temperature" of Gunn and Cosway (1938). It is distinct from the "Vorzugstemperatur" used by Herter (1940) and modified by Bogert (1949) to "preferred substrate temperature" (PST).

Little is known about thermoregulation in salamanders. Although Brattstrom (1963) states that "most salamanders have no 'preferred temperature,'" it is possible that many species behaviorally regulate body temperatures by selection of appropriate microhabitats. These experiments were conducted to determine if the species studied employed behavioral thermoregulation, and, if so, whether their preferred temperatures differed. Salamanders were exposed to a substrate thermal gradient encompassing temperatures extending well beyond the limits within which they are usually found in the field.

Methods

Gradient design.—The temperature gradient was established along a steel bar 182.9 by 15.2 by 0.6 cm supported within a wooden box. The selection chamber measured 152.4 by 15.2 by 7.6 cm and was formed by the steel bar on the bottom, a wood top and sides, and wooden partitions at each end. The gradient was maintained by immersing one end of the bar in an ice bath and placing a heating element controlled with a variable transformer on the other end. The gradient box was insulated on all sides by 1-inch styrofoam and was maintained in a laboratory where the ambient temperature fluctuated between 21° and 27°C.

Temperature was monitored by 31 thermistor probes (Yellow Springs Instruments, YSI 401) taped to the top of the bar at 2-inch intervals and connected via switch boxes to YSI telethermometers (Models 42SC and 44TZ). Readings were accurate to $\pm 0.2^\circ\text{C}$. Average floor temperatures ranged from -0.2° to 53.2°C . The gradient was approximately linear with temperature increments of about 0.5° – 1.0°C between the majority of the stations. Temperature differences between stations were more pronounced near the hot and cold ends. A typical temperature profile is shown in Fig. 4.

The steel bar was covered with a $\frac{1}{4}$ -inch layer of wet vermiculite which served as a moist, uniform, inert substrate. When the top of the gradient was

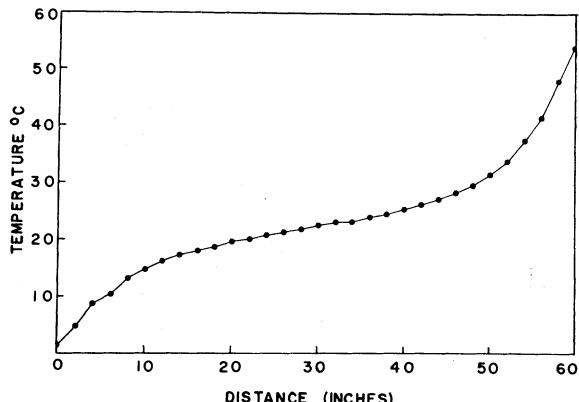


FIG. 4. A typical temperature profile of the floor of the thermal gradient. Each point is the mean of eight readings taken on 2 days.

closed the selection chamber remained saturated with moisture. Diffuse lighting was provided during the light portions of the experimental photoperiods by overhead fluorescent lamps in the room. A double layer of black cloth was placed over the top of the gradient during the dark portions of these photoperiods.

Testing procedure.—Groups of up to 11 individuals were placed in the selection chamber and after approximately 2 hr gradient temperatures were recorded and positions of salamanders noted. Preliminary experiments indicated that the positions of salamanders stabilized within 1 hr after insertion into the chamber. No intra- or interspecific interactions were observed among the salamanders. The number of animals placed into the gradient did not appear to affect the temperatures selected by individuals. No individual was tested in the gradient more than two times. Tests were conducted between 0730 and 2330 CST. The same group of animals was never used during two consecutive test periods.

In this experiment the preferred substrate temperature (PST) of the salamanders was determined rather than the preferred body temperature (PBT). In heliothermic reptiles the PST is not an accurate indicator of the PBT (Bogert 1949). Temperatures of terrestrial salamanders found in a moist microhabitat are usually the same as the substrate temperature (Bogert 1952, Cunningham 1960, Brattstrom 1963, Vial 1968). Since the selection chamber provided a small, moist, enclosed microhabitat with little air movement, the substrate temperatures recorded for the salamanders were assumed to be essentially the same as their body temperatures.

Results

Thermal response.—When first put into the selection chamber, salamanders actively explored it for up to 1 hr. They then tended to occupy stable positions at intermediate temperatures. In preliminary experi-

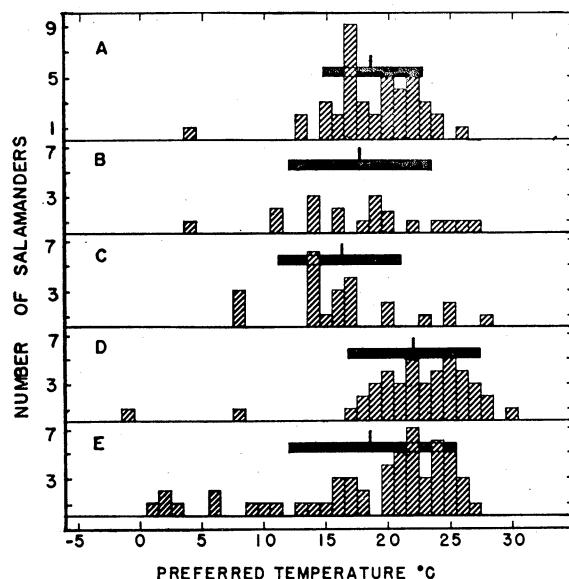


FIG. 5. Preferred temperatures of *P. ouachitae* acclimated at 5°C (LD 16:8 and LD 8:16) (A), *P. c. serratus* (B) and *P. jordani* (D) and *P. glutinosus* (Ozark) (E) at 15°C (LD 16:8), and *P. jordani* (C) at 25°C (LD 16:8 and 8:16). Vertical bars represent the number of individuals at each temperature. Vertical lines represent the mean and horizontal bars one SD of the observations.

ments animals placed in the selection chamber, with no temperature gradient applied, distributed themselves all along its length, but showed a tendency to congregate at the ends of the chamber. The size of the test group did not affect this trend, nor did the number of salamanders in the gradient. When the gradient was in effect salamanders avoided the hot end and tended to go toward the cold end. This tendency skewed the results by extending the preferred temperature (PT) ranges toward 0°C.

The temperature selection of five species of salamanders is shown in Fig. 5. Distributions were clumped in a small portion of the temperature range of the gradient. Individuals aggregated within a small range of temperatures around the mean rather than being uniformly distributed throughout the range. This indicated that these species were selecting a definite range of preferred temperatures and were not merely avoiding temperature extremes. The standard deviation (sd) appeared to be a good indicator of the thermal preferendum of a species. All of the species tested had the same type of clumped distribution.

Preferred temperatures.—Salamanders in groups I and II had stable thermal preferences that were not affected by acclimation temperature or photoperiod. The four populations in the first test group had similar preferred temperatures (Fig. 6). No definite trend, such as that for CTM, was apparent. The PT

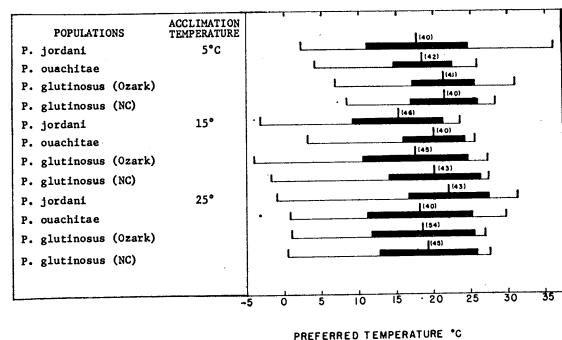


FIG. 6. Preferred temperatures of *P. jordani*, *P. ouachitae*, *P. glutinosus* (Ozark), and *P. glutinosus* (NC) acclimated to three temperatures and two photoperiods. The results for the two light regimes at each temperature are pooled into one sample. See Fig. 1 for explanation of symbols.

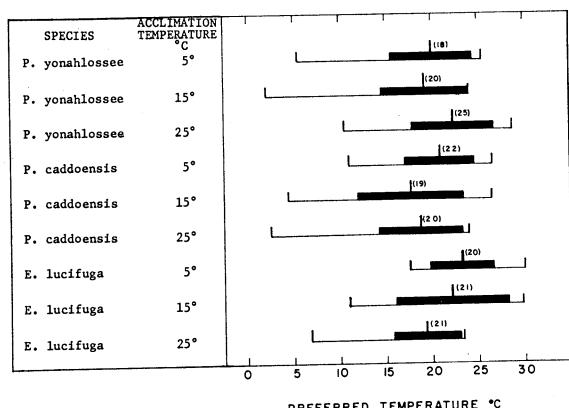


FIG. 7. Preferred temperatures of *P. yonahlossee*, *P. caddoensis*, and *E. lucifuga* acclimated to 5°, 15°, and 25°C (LD 16:8). See Fig. 1 for explanation of symbols.

values for species in group II (Fig. 7) were significantly different ($P \leq 0.05$). *Plethodon yonahlossee* had the lowest PT (20.2°C) when acclimated to 5°C and the highest (22.4°C) when acclimated to 25°C. The PT of *E. lucifuga* decreased as the acclimation temperature increased. *Plethodon caddoensis* had the lowest PT (17.7° and 18.8°C) when acclimated to 15°C and 25°C.

The differences in thermal preference of the 13 populations of group III were highly significant ($P \leq 0.01$). These differences were clarified by Duncan's new multiple range test (Table 2). The two populations of *D. fuscus* had the highest mean PT values (Rich Mountain, 23.8°C and Albert Pike, 25.6°C), and the small plethodonts *P. dorsalis*, *P. c. cinereus*, and *P. c. serratus* had the lowest means (14.4°C, 16.2°C, and 16.3°C respectively). The PT of *E. longicauda* was significantly different from that of *P. dorsalis*, *P. c. serratus*, and *P. glutinosus* (Ouachita), but was similar to that of *E. multiplicata* (Fig. 8).

There was a progressive increase in the PT of the

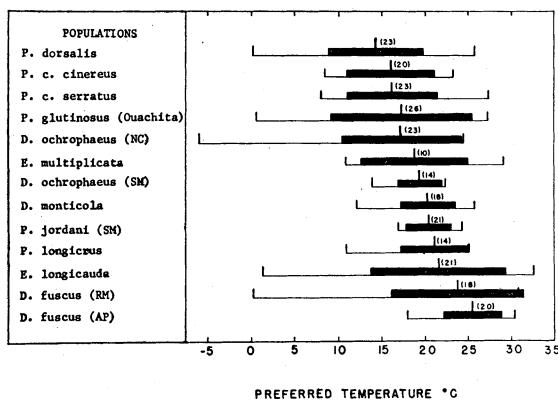


FIG. 8. The preferred temperatures of 13 populations of salamanders acclimated to 15°C (LD 16:8). See Fig. 1 for explanation of symbols.

TABLE 2. Mean preferred temperature (PT) of 13 populations of salamanders acclimated to 15°C (LD 16:8) (group III)

Population	Number of observations	PT (°C) ^a
P. dorsalis	23	14.4
P. c. cinereus	20	16.2
P. c. serratus	23	16.3
P. glutinosus (Ouachita)	26	17.4
D. ochrophaeus (NC)	23	17.4
E. multiplicata	10	18.8
D. ochrophaeus (SM)	14	19.5
D. monticola	18	20.3
P. jordani (SM)	21	20.5
P. longicrus	14	21.2
E. longicauda	21	21.7
D. fuscus (RM)	18	23.8
D. fuscus (AP)	20	25.6

*Any two means not encompassed by the same vertical line are significantly different as determined by Duncan's new multiple range test.

salamanders exposed to all six acclimation states (group I) as time of day shifted from 0730 to 2330 CST. The β estimate from the ANCOVA was $0.0359^{\circ}\text{C hr}^{-1}$ with a t -value of 3.74 ($P = 0.000$; 47 df). The time of day had no effect on the PT of the salamanders in the other groups.

DISCUSSION

Stability of thermal preferences.—The responses of salamanders in the temperature gradient were not consistent with the view of Brattstrom (1963) that these animals have no preferred temperatures. All species tested exhibited well-defined thermal preferences under all acclimation conditions and thus showed some capacity for behavioral thermoregulation.

Thermal preferences were relatively stable for each species and were not significantly affected by either acclimation temperature or photoperiod. Licht and Brown (1967) reported that acclimation to 5°, 15°, and 30°C for 18 days had little effect on the

PT of *Taricha rivularis* (Twitty). The PT of *Anolis carolinensis* Voigt was not affected by thermal acclimation or day length (Licht 1968). Apparently the PT is independent of past thermal history in these species. Wilhoft and Anderson (1960) reported a drop in the PT of *Sceloporus occidentalis* Baird and Girard after acclimation to high temperatures. This may be an unusual reaction due to the high acclimation temperature (35°C) used in their study or may represent a more labile PT than that seen in *A. carolinensis*. Lucas and Reynolds (1967) reported a drop in the PT of *Rana pipiens* larvae reared at both low (13°C) and high ($25^{\circ}\text{--}27^{\circ}\text{C}$) temperatures as compared to control larvae reared at 20°C . The thermal preferences of fish are very labile, pronounced shifts occurring in response to small changes in acclimation temperature (Norris 1963, Fry 1964). There appear to be interspecific differences in the stability of the thermal preferences among poikilothermic vertebrates. Salamanders and, according to Licht (1968), lizards have stable thermal preferences, whereas frog larvae and fish have relatively labile thermal preferences.

Interspecific differences.—In general the thermal preferences of the species in this study were not as precise as those reported for *Taricha* larvae and adults (Licht and Brown 1967). Apparently newts have evolved a more precise behavioral thermoregulation than plethodontid salamanders. Preferred temperatures differed in congeneric species of plethodontid salamanders. Licht et al. (1966) reported that PT was similar in congeneric species of Australian lizards, and Licht and Brown (1967) reported that temperature preferenda were the same among larval newts of the genus *Taricha*. The less precise behavioral thermoregulation of plethodontid salamanders may be more readily altered by diverse environmental temperatures in different habitats. Thus *D. fuscus* from Camp Albert Pike had a significantly higher preferred temperature than members of the genus *Desmognathus* from the cool mountains of North Carolina, and *P. longicrus* from the valley at Bat Caves, N.C., had a significantly higher PT than *P. c. cinereus* from Grandfather Mountain (Fig. 8).

Differences also appeared at the generic level. *Eurycea lucifuga* had a higher thermal preference than *P. caddoensis* (Fig. 7), and *E. longicauda* had a higher PT than the Arkansas plethodonts in group III (Fig. 8). Allopatric populations within the same species had similar thermal preferences. This was evident for *P. cinereus*, *D. fuscus*, *D. ochrophaeus*, and *P. glutinosus* (Fig. 6 and 8).

Optimum temperature.—Whitford and Hutchison (1965b) stated that the ability of amphibian species to obtain sufficient oxygen at higher temperatures may determine their optimum temperature. They compared the rates of pulmonary (including bucco-

pharyngeal) and cutaneous respiration in several species at 15°C and found that *T. granulosa* (Skilton) obtained about 50% of its oxygen through pulmonary respiration, whereas *P. glutinosus* obtained 24.4%, *D. monticola* 18.7%, and *D. quadramaculatus* (Holbrook) 15.2% of their oxygen through buccopharyngeal respiration. They concluded that the increased efficiency of the breathing mechanism of lunged salamanders at higher temperatures may account for their ability to function at these temperatures.

A comparison of the thermal preferences of terrestrial juvenile and adult *T. rivularis* (Licht and Brown 1967) with those of *P. glutinosus* shows that the former species has a PT range of about 15°–27°C, whereas *P. glutinosus* has a thermal preference of 9°–27°C (Fig. 6, 7, and 8). The mean value for the juvenile *T. rivularis* was about 22°C while the mean values for *P. glutinosus* ranged from 17.4°C to 21.7°C. Although the respiratory exchanges of lunged salamanders are more efficient at higher temperatures, their preferred temperatures may be only slightly higher than those of the lungless forms. The main difference between *T. rivularis* and *P. glutinosus* was the lack of selection of temperatures below 15°C by the former species. Likewise, though *P. glutinosus* may have more efficient buccopharyngeal respiration than *D. ochrophaeus* and *D. monticola*, their preferred temperatures were similar. Whitford and Hutchison (1967) suggested that the optimum metabolism of both the lunged salamander *Ambystoma maculatum* (Shaw) and the lungless species *D. quadramaculatus* occurred between 15° and 20°C. The stable thermal preferenda of the species I tested may be a reflection of their optimum temperature for metabolic efficiency.

Salamanders in group I (all six acclimation states) preferred higher temperatures at night than during the day. Licht and Brown (1967) reported the opposite reaction for some adult *T. rivularis* that exhibited a nocturnal shift to lower temperatures. Time of day had no significant effect on the preferred temperature of group II and group III salamanders; the significance of the response of the group I animals is not understood.

DEHYDRATION

Terrestrial salamanders and free water surfaces of equal area lose water through evaporation at similar rates (Spight 1968). Unlike frogs they do not exhibit any change in skin permeability to water due to hormonal action (Bentley and Heller 1964, Alvarado and Johnson 1965). The small interspecific differences in water-loss rates among plethodontid salamanders is apparently due to differences in their body shapes (MacMahon 1965, Spight 1968).

This series of experiments was conducted to de-

termine the effects of vapor pressure deficit (VPD) and temperature on the rate of water loss in plethodontid salamanders. The influence of these parameters on the rate of water loss is an important factor in the success of a species in a particular habitat.

Methods

Two separate experiments were conducted. The first was conducted on all species under all acclimation conditions (set A). Salamanders were dehydrated in still air. Most previous studies on water loss in amphibians have used moving air (Thorson and Svhil 1943, Littleford et al. 1947, Cohen 1952, Gordon 1952, Thorson 1955, 1956, Hutchison 1958, Ray 1958, Schmid 1965). Still air allowed a slow dehydration similar to that caused by conditions in the natural microhabitat where air movement is at a minimum during times of salamander activity.

Salamanders were dried with paper towels and their bladders emptied by pressing down on the dorsal side of the pelvic girdle. They were then weighed, put into small screen wire cages, and placed into 8-inch desiccators in groups of up to seven individuals. Desiccators were placed in the environmental chamber and maintained at the appropriate acclimation condition (test temperature = acclimation temperature). Desiccators were returned to the laboratory, and the salamanders were removed and weighed at regular intervals (usually 2, 4, 6, and 8 hr) until they lost approximately 5–10% of their initial body weight. Salamanders were not prevented from coiling in the cages. Attempts were made to prevent any weight loss greater than 12–15% to eliminate serious injury to the salamanders and minimize physiological imbalance that might affect the results of the other tests. After completing dehydration, salamanders were rehydrated in small culture dishes partially filled with water. All tests were conducted between 0750 and 1825 CST.

Results were expressed as weight loss (respiratory surface area) $^{-1}$ hour $^{-1}$ in mg $^{-1}$ cm $^{-2}$ hr $^{-1}$. Respiratory surface area (RSA) was used rather than surface area because salamanders lose water not only through the skin but also from the mouth and buccal cavity. The RSA value was determined with the formula of Whitford and Hutchison (1967): $\log S = 0.983 + 0.614 \log W$, or $S = 9.62 W^{0.614}$, where S = RSA in square centimeters and W = weight in grams. Data were not used if an animal urinated during the test period. Tolerance to desiccation was expressed as a rate function; death was not used as a measure of the vital limit of exsiccation because it could be due to some factor other than dehydration.

Desiccators contained anhydrous CaSO₄. With salamanders in a desiccator, the relative humidity (RH) was 80.0–88.0% at 5°C (VPD 0.8–1.3 mm Hg), 65–73% at 15°C (VPD 3.45–4.48 mm Hg),

TABLE 3. Specific gravity, expected and actual relative humidity (RH), and vapor pressure deficit (VPD) of glycerol-water solutions used to maintain desired levels of water vapor in dehydration jars

Temperature (°C)	Specific gravity	Expected RH ^a (%)	Actual RH (%)	Vapor pressure deficit (mm Hg)
5	1.198	60	58.3–63.5	2.4–2.7
	1.163	70	68.5–70.0	2.0–2.1
	1.137	80	77.7–78.5	1.4–1.5
	1.098	90	87.3–89.0	0.7–0.8
	1.000	100	Saturated	0
15	1.198	60	58.5–62.7	4.8–5.3
	1.163	70	66.2–73.5	3.4–4.3
	1.137	80	77.5–80.5	2.5–2.9
	1.098	90	86.5–89.0	1.2–1.4
	1.000	100	Saturated	0
25	1.178	60	60.0–64.0	8.6–9.5
	1.165	70	66.5–70.0	7.1–8.0
	1.137	80	77.5–80.0	4.8–5.4
	1.098	90	86.0–90.0	2.4–3.3
	1.000	100	Saturated	0

^aExpected RH was computed with data from Braun and Braun (1958)

and 62.0–66.0% at 25°C (VPD 8.1–9.0 mm Hg). These RH readings were taken just above the desiccator plate within 1–2 inches of the salamanders. Measurements were made with an electric hygrometer indicator (Hydrodynamics Inc., model 15–3001) with narrow range sensing elements. This system has an accuracy of $\pm 1.5\%$ RH.

Actually the CaSO_4 creates a relative humidity of 0–5% in the air adjacent to it, and the RH at the CaSO_4 -air interface was probably very close to 0%. Salamanders in the desiccator constantly lose water from their respiratory surfaces so that the RH at the respiratory surface-air interface was probably 95–100% (VPD 0.0–0.3 mm Hg at 5°C, 0.0–0.6 mm Hg at 15°C, and 0.0–1.2 mm Hg at 25°C). Thus a moisture gradient is created in the desiccator from the respiratory surface of the salamanders to the surface of the CaSO_4 . The air close to the salamander has a high RH and low VPD and that near the CaSO_4 has a low RH and high VPD. The rate of water loss from the salamander is dependent upon the steepness of the moisture gradient from the surface of the salamander to that of the CaSO_4 .

The second set of experiments (set B) was also conducted on individuals of all species under all acclimation conditions. Salamanders were dehydrated at 60%, 70%, 80%, 90%, and 100% RH at 5°, 15°, and 25°C. These RH values represent VPD's of 2.6, 2.0, 1.3, 0.6, and 0.0 mm Hg at 5°C; 5.1, 3.8, 2.6, 1.3, and 0.0 mm Hg at 15°C; and 9.5, 7.1, 4.8, 2.4, and 0.0 mm Hg at 25°C.

Solutions of reagent grade glycerol and distilled water were prepared in various concentrations calculated to produce the desired levels of water vapor pressure (Johnson 1940, Braun and Braun 1958).

Specific gravity, expected RH, actual RH, and VPD of these solutions are given in Table 3. Specific gravities were measured with a hygrometer. Salamanders placed into these solutions for short periods of time (1–5 min) suffered no visible adverse effects. Animals left in the more concentrated solutions for 1–2 hr died from excess water loss.

Salamanders were dried and weighed as in the other dehydration experiment and placed into 450-ml jars over the glycerol-water solutions. Jars were returned to the environmental chamber at the appropriate acclimation condition, and after 4 hr (1–2 hr for very small individuals) the salamanders were removed, weighed, and placed in soil to rehydrate. Results were expressed as weight loss (respiratory surface area) $^{-1}$ time $^{-1}$ in mg cm^{-2} (4 hrs) $^{-1}$. Data were not used if an animal urinated during the experimental period. All tests were conducted between 0740 and 1930 CST (test temperature = acclimation temperature). The moisture gradient in each jar from the salamander to the glycerol solution was not as steep as that in the desiccators because of the lower VPD created by the glycerol solutions.

All weights in the dehydration and rehydration experiments were recorded to 0.01 ± 0.005 g. In the early part of the study a torsion balance accurate to ± 0.005 g was used, but for the majority of the experiments a Mettler top-loading balance (model P-120) accurate to ± 0.002 g was employed.

Results

Dehydration in dry air.—Dehydration rates in the desiccators (set A experiments) were dependent upon the weight of the salamander, its species, and the acclimation temperature. The effect of weight was highly significant for the animals exposed to all six acclimation states (group I). The β estimate from the ANCOVA was $-0.236 \text{ mg cm}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ with a t -value of -4.36 ($P = 0.000$; 47 df). Weight also had a significant effect on the dehydration rate of the 13 groups of salamanders tested at 15°C (group III). The β estimate was $-0.204 \text{ mg cm}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ with a t -value of -2.47 ($P \leq 0.015$; 12 df). The rates of dehydration of *P. yonahlossee*, *P. caddoensis*, and *E. lucifuga* (group II) were not significantly affected by their weight.

Dehydration rates of the salamanders in group I increased with increased acclimation temperature (Fig. 9). The means for all individuals at 5°, 15°, and 25°C were $3 \text{ mg cm}^{-2} \text{ hr}^{-1}$, $5 \text{ mg cm}^{-2} \text{ hr}^{-1}$, and $7 \text{ mg cm}^{-2} \text{ hr}^{-1}$. The temperature effect was highly significant ($P \leq 0.01$), but population differences were not apparent.

The rates of dehydration of the animals in group II were significantly affected by both temperature and species ($P \leq 0.01$). Dehydration rates ranged from $2 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 5°C to $9 \text{ mg cm}^{-2} \text{ hr}^{-1}$

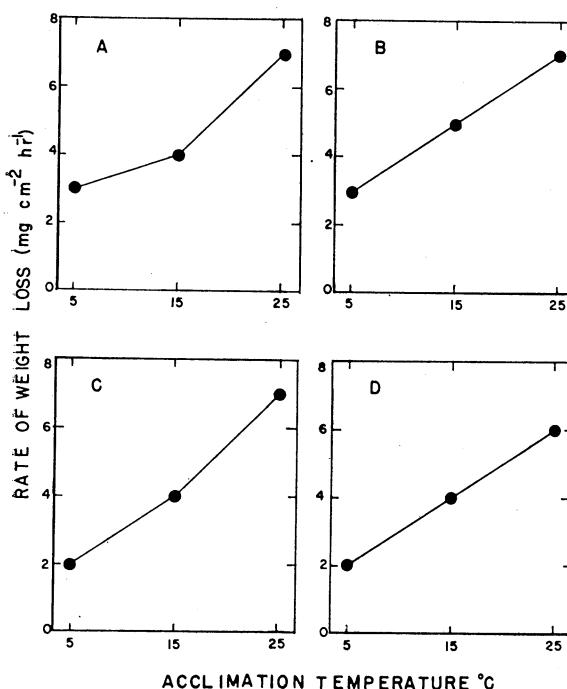


FIG. 9. The rate of dehydration in dry air of *P. jordani*, *P. ouachitae*, *P. glutinosus* (Ozark), and *P. glutinosus* (NC) acclimated to three temperatures and two photoperiods. The results for the two light regimes at each temperature are pooled into one sample. (A) *P. jordani*; (B) *P. ouachitae*; (C) *P. glutinosus* (Ozark); (D) *P. glutinosus* (NC).

for *P. caddoensis* at 15°C. *Plethodon caddoensis* had the highest dehydration rate followed by *E. lucifuga* and *P. yonahlossee* (Fig. 10).

Differences were highly significant ($P \leq 0.01$) in the rates of dehydration of the 13 populations (group III) acclimated and tested at 15°C (LD 16:8) (Table 4). *Desmognathus fuscus* and *P. glutinosus* (Ouachita) had the lowest rate ($3 \text{ mg cm}^{-2} \text{ hr}^{-1}$), and *P. c. serratus* had the highest ($10 \text{ mg cm}^{-2} \text{ hr}^{-1}$). Rate differences appeared to be due to size differences among the species.

Water loss at high relative humidities.—At high relative humidities (set B experiments) the rate of dehydration decreased as the relative humidity increased from 60% to 100%. The ANCOVA for the effect of RH on the rate of dehydration was highly significant. The β estimate was $-0.238 \text{ mg cm}^{-2} (4 \text{ hr})^{-1}$ per cent relative humidity $^{-1}$ with a t -value of -23.33 ($P = 0.000$; 45 df). The μ estimate was 25.95. A representative graph, prepared from the regression equations for *P. jordani* (NC), is presented in Fig. 11. The regression equations for all of the species tested under all conditions are presented in Table 5. The regressions for the salamanders tested at 5°, 15°, and 25°C show the effect of temperature on the rate of dehydration. At higher temperatures the VPD increased and the rate of de-

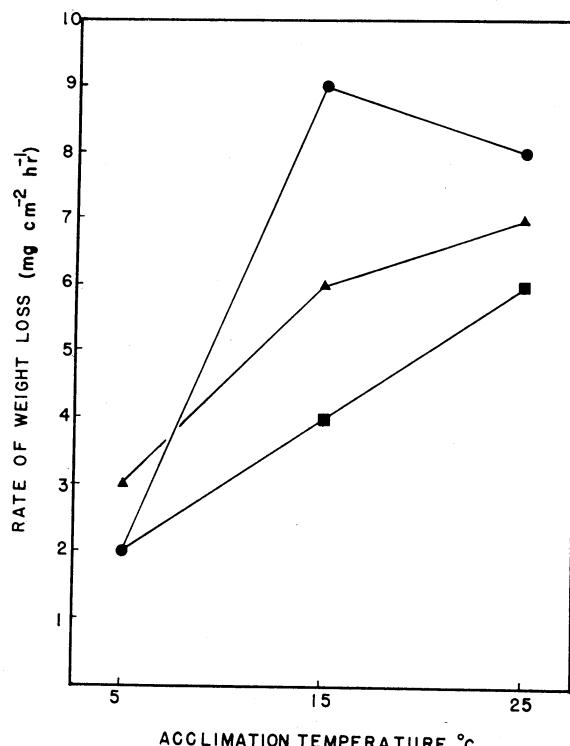


FIG. 10. Dehydration rates in dry air of *P. yonahlossee* (■—■), *P. caddoensis* (●—●), and *E. lucifuga* (▲—▲) acclimated to 5°, 15°, and 25°C (LD 16:8).

TABLE 4. Mean rate of dehydration in dry air of 13 populations of salamanders acclimated to 15°C (LD 16:8) (group III)

Population	Number of observations	Mean rate ^a ($\text{mg cm}^{-2} \text{ hr}^{-1}$)
<i>D. fuscus</i> (RM)	10	3
<i>P. glutinosus</i> (Ouachita)	15	3
<i>D. fuscus</i> (AP)	10	3
<i>P. jordani</i> (SM)	10	4
<i>P. longicrus</i>	6	4
<i>D. ochrophaeus</i> (SM)	7	4
<i>E. longicauda</i>	12	5
<i>D. monticola</i>	11	5
<i>D. ochrophaeus</i> (NC)	13	5
<i>P. dorsalis</i>	13	5
<i>E. multiplicata</i>	6	6
<i>P. c. cinereus</i>	10	7
<i>P. c. serratus</i>	15	10

^aAny two means not encompassed by the same vertical line are significantly different as determined by Duncan's new multiple range test.

hydration increased accordingly. Differences among the species in group I were not statistically significant. At all three temperatures the dehydration rates of *P. jordani*, *P. ouachitae*, and *P. glutinosus* (NC) were similar.

At high relative humidities *P. caddoensis* dehydrated faster than *P. yonahlossee* (group II). *Plethodon c. serratus* had the highest dehydration rate ($13.9 \text{ mg cm}^{-2} (4 \text{ hr})^{-1}$ at 60% RH); *D. fuscus*

TABLE 5. Regression equations for the rate of weight loss of salamanders dehydrated at high relative humidities (Y = rate of weight loss and RH = relative humidity)

Population	Acclimation condition	Regression equation
<i>P. jordani</i> (NC)	5°C (LD 16:8)	$Y = 24.78 - 0.24 RH$
	5°C (LD 8:16)	$Y = 22.58 - 0.24 RH$
	15°C (LD 16:8)	$Y = 24.99 - 0.24 RH$
	15°C (LD 8:16)	$Y = 24.08 - 0.24 RH$
	25°C (LD 16:8)	$Y = 30.36 - 0.24 RH$
	25°C (LD 8:16)	$Y = 29.22 - 0.24 RH$
<i>P. ouachitae</i>	5°C (LD 16:8)	$Y = 23.05 - 0.24 RH$
	5°C (LD 8:16)	$Y = 23.22 - 0.24 RH$
	15°C (LD 16:8)	$Y = 25.58 - 0.24 RH$
	15°C (LD 8:16)	$Y = 25.39 - 0.24 RH$
	25°C (LD 16:8)	$Y = 28.28 - 0.24 RH$
	25°C (LD 8:16)	$Y = 31.60 - 0.24 RH$
<i>P. glutinosus</i> (Ozark)	5°C (LD 16:8)	$Y = 23.68 - 0.24 RH$
	5°C (LD 8:16)	$Y = 22.68 - 0.24 RH$
	15°C (LD 16:8)	$Y = 25.25 - 0.24 RH$
	15°C (LD 8:16)	$Y = 24.71 - 0.24 RH$
	25°C (LD 16:8)	$Y = 28.50 - 0.24 RH$
	25°C (LD 8:16)	$Y = 29.82 - 0.24 RH$
<i>P. glutinosus</i> (NC)	5°C (LD 16:8)	$Y = 23.48 - 0.24 RH$
	5°C (LD 8:16)	$Y = 23.28 - 0.24 RH$
	15°C (LD 16:8)	$Y = 24.97 - 0.24 RH$
	15°C (LD 8:16)	$Y = 22.98 - 0.24 RH$
	25°C (LD 16:8)	$Y = 29.90 - 0.24 RH$
	25°C (LD 8:16)	$Y = 29.85 - 0.24 RH$
<i>P. yonahlossee</i>	5°C (LD 16:8)	$Y = 23.18 - 0.24 RH$
	15°C (LD 16:8)	$Y = 25.11 - 0.24 RH$
	25°C (LD 16:8)	$Y = 26.45 - 0.24 RH$
<i>P. caddoensis</i>	5°C (LD 16:8)	$Y = 23.68 - 0.24 RH$
	15°C (LD 16:8)	$Y = 25.63 - 0.24 RH$
	25°C (LD 16:8)	$Y = 29.58 - 0.24 RH$
<i>E. lucifuga</i>	5°C (LD 16:8)	$Y = 25.25 - 0.24 RH$
	15°C (LD 16:8)	$Y = 25.73 - 0.24 RH$
	25°C (LD 16:8)	$Y = 33.48 - 0.24 RH$
<i>P. c. cinereus</i>	15°C (LD 16:8)	$Y = 25.08 - 0.24 RH$
<i>P. c. serratus</i>	15°C (LD 16:8)	$Y = 28.30 - 0.24 RH$
<i>P. dorsalis</i>	15°C (LD 16:8)	$Y = 26.03 - 0.24 RH$
<i>P. glutinosus</i> (Ouachita)	15°C (LD 16:8)	$Y = 24.63 - 0.24 RH$
<i>P. jordani</i> (SM)	15°C (LD 16:8)	$Y = 24.88 - 0.24 RH$
<i>P. longicrus</i>	15°C (LD 16:8)	$Y = 24.66 - 0.24 RH$
<i>D. fuscus</i> (AP)	15°C (LD 16:8)	$Y = 23.78 - 0.24 RH$
<i>D. fuscus</i> (RM)	15°C (LD 16:8)	$Y = 22.78 - 0.24 RH$
<i>D. monticola</i>	15°C (LD 16:8)	$Y = 25.50 - 0.24 RH$
<i>D. ochrophaeus</i> (NC)	15°C (LD 16:8)	$Y = 26.87 - 0.24 RH$
<i>D. ochrophaeus</i> (SM)	15°C (LD 16:8)	$Y = 25.51 - 0.24 RH$
<i>E. longicauda</i>	15°C (LD 16:8)	$Y = 26.75 - 0.24 RH$
<i>E. multiplicata</i>	15°C (LD 16:8)	$Y = 26.18 - 0.24 RH$

(RM) had the lowest rate (8.4 mg cm^{-2} (4 hr) $^{-1}$ at 60% RH) of the salamanders tested only at 15°C (group III). Species differences appeared to be size dependent. Photoperiod had no significant effect in these experiments.

Discussion

Effect of size.—The rate of dehydration in these species is dependent upon body weight, the drying power of the air, and ambient temperature. Large salamanders dehydrate at a slower rate than small ones. The interspecific differences in dehydration rates

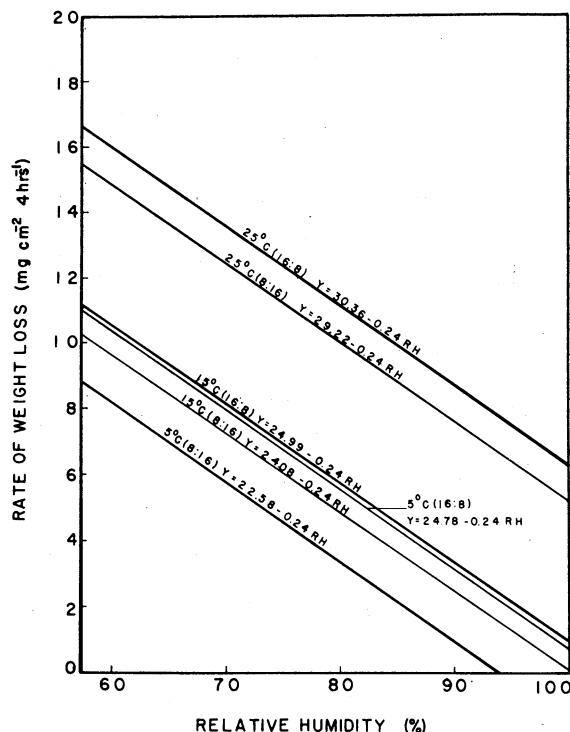


FIG. 11. Regressions for the rate of dehydration at high relative humidities of *P. jordani* acclimated to three temperatures and two photoperiods.

appear to be related in part to weight differences. *Plethodon c. serratus* (average weight 0.62 g) lost water at a rate of $10 \text{ mg cm}^{-2} \text{ hr}^{-1}$, whereas *P. glutinosus* (Ouachita) (average weight 3.93 g) lost water at a rate of $3 \text{ mg cm}^{-2} \text{ hr}^{-1}$. Surface configuration also affected dehydration rates. Spight (1968) reported that equal areas of surface on large salamanders lost water at a slower rate than those on small salamanders because of differences in size and shape of the animals. MacMahon (1964) demonstrated that small interspecific differences in the dehydration rates of salamanders of the genus *Plethodon* were related to differences in their body shapes. All of the species considered in this study would experience similar rates of water loss if exposed to uniform environmental conditions. Major differences would relate to size differences so that species composed of small individuals would lose water faster than species made up of large individuals.

Effect of VPD and temperature.—Rate of water loss was directly related to vapor pressure deficit. As the VPD increased, the dehydration rate increased. *Plethodon ouachitae* at 15°C dehydrated at a rate of $5 \text{ mg cm}^{-2} \text{ hr}^{-1}$ in dry air, $2.8 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 60% RH (VPD 4.8–5.3 mm Hg), and $1 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 90% RH (VPD 1.2–1.4 mm Hg). This effect has been noted in salamanders by Mac-

Mahon (1964), in frogs by Adolph (1932), Jameson (1966), and Warburg (1967), and in lizards by Warburg (1965a, 1966).

Temperature has a direct effect on the dehydration rate of salamanders. An increase in temperature raises the VPD of the air and causes more rapid dehydration. Salamanders dehydrate at similar rates when exposed to the same VPD at different temperatures. *Plethodon ouachitae* dehydrated at a rate of $1 \text{ mg cm}^{-2} \text{ hr}^{-1}$ when exposed to a VPD of $1.4\text{--}1.5 \text{ mm Hg}$ at 5°C and $1.2\text{--}1.4 \text{ mm Hg}$ at 15°C . When exposed to a VPD of $2.4\text{--}3.3 \text{ mm Hg}$ it lost $2.2 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 5°C , $1.6 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 15°C , and $2.1 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 25°C . The increased dehydration rates of salamanders in the desiccators at higher temperatures were probably due to an increase in the steepness of the moisture gradient from the salamander to the CaSO_4 and an increased metabolic rate of the salamanders at these temperatures. The dehydration curves for *Plethodon* salamanders in dry air at 5° , 15° , and 25°C (Fig. 9) are similar in shape to that for the metabolic rate of *D. quadramaculatus* at the same temperatures presented by Whitford and Hutchison (1967:129). The Q_{10} for the metabolic rate of *D. quadramaculatus* from 5° to 25°C calculated from their graph (1.62) is similar to the Q_{10} for the pooled dehydration rates from 5° to 25°C (1.66) for the salamanders in Fig. 9.

Influence of behavior.—These salamanders do not appear to have any physiological control over their rate of dehydration. MacMahon (1965) found that agar models of salamanders with the same shape as their living counterparts had the same rate of water loss. Spight (1968) stated that circulation was not necessary to maintain the water supply to the skin when an amphibian was in still air and suggested that water was freely available at the amphibian skin for evaporation. Heatwole et al. (1969) and Gehlbach, Kimmel, and Weems (1969) have demonstrated that behavioral adjustments are important in reducing the dehydration rates in frogs and salamanders. Salamanders assumed partially coiled positions when exposed to high VPD and high temperatures, but this behavior had a minimal effect on the dehydration rate because individuals were unable to coil tightly in the cages and could not form aggregations.

REHYDRATION

The rate at which salamanders take up water through their skin is an important factor in their ability to survive dehydration associated with normal surface activity and prolonged periods of moisture stress underground. Interspecific differences in rehydration rates have been reported for both frogs (Ewer 1952, Thorson 1955, Bentley, Lee, and Main 1958, Main and Bentley 1964, Warburg 1965b, 1967) and salamanders (Cohen 1952, Spight 1967b).

In this series of experiments salamanders were rehydrated in water to determine the effects of temperature, dehydration deficit, time, and species on the rate of water uptake.

Methods

Salamanders were dehydrated in desiccators until they lost 5–10% of their body weight (dehydration experiment). They were then placed into 4-inch culture dishes containing approximately 1 cm of tap water. Dishes were covered and placed in the environmental chamber at the appropriate acclimation condition. Dishes were returned to the laboratory at regular intervals (usually 2, 4, 6, and 8 hr), and the salamanders were removed, dried in paper towels, and weighed. The bladder was not emptied before the salamander was weighed. Data were not used if an animal urinated between weighings. All experiments were conducted between 0835 and 2330 CST. Test temperatures were the same as acclimation temperatures. Since salamanders were never observed taking up water via their mouths, results were recorded as weight gain ($\text{surface area})^{-1} \text{ hour}^{-1}$ in $\text{mg cm}^{-2} \text{ hr}^{-1}$. Surface areas were calculated with the formula of Whitford and Hutchison (1967): $\log S = 0.924 + 0.694 \log W$, or $S = 8.42 W^{0.694}$, where S = surface area in square centimeters and W = weight in grams.

Results

Effect of weight and weight loss.—Rate of rehydration in salamanders in group I (all six acclimation states) and group II (three temperatures) increased as the original weight and percentage weight loss became larger. For group I the ANCOVA gave a β_1 estimate for weight of $0.003 \text{ mg cm}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ with a t -value of 3.56 ($P = 0.000$; 47 df) and a β_2 estimate for weight loss of $0.001 \text{ mg cm}^{-2} \text{ hr}^{-1} \%^{-1}$ with a t -value of 3.11 ($P \leq 0.002$; 47 df). For group II the β_1 estimate for weight was $0.464 \text{ mg cm}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ with a t -value of 3.17 ($P \leq 0.002$; 8 df), and the β_2 estimate for weight loss was $0.259 \text{ mg cm}^{-2} \text{ hr}^{-1} \%^{-1}$ with a t -value of 2.94 ($P \leq 0.004$; 8 df). Increased weight loss had a highly significant effect on the rate of rehydration in group III salamanders acclimated to 15°C as demonstrated by the ANCOVA. The β_2 estimate for weight loss was $0.175 \text{ mg cm}^{-2} \text{ hr}^{-1} \%^{-1}$ with a t -value of 2.76 ($P \leq 0.007$; 12 df). Rehydration rate was not significantly affected by the weight of the salamanders in group III.

Time and temperature effects.—Time period (0–2, 2–4, and 4–6 hr) had a highly significant effect on the rate of rehydration. The ANCOVA indicated that the regression for time period was quadratic. The model equation was:

$$Y = \mu + \alpha_1 + \beta_1 X + \beta_2 X^2 + \epsilon,$$

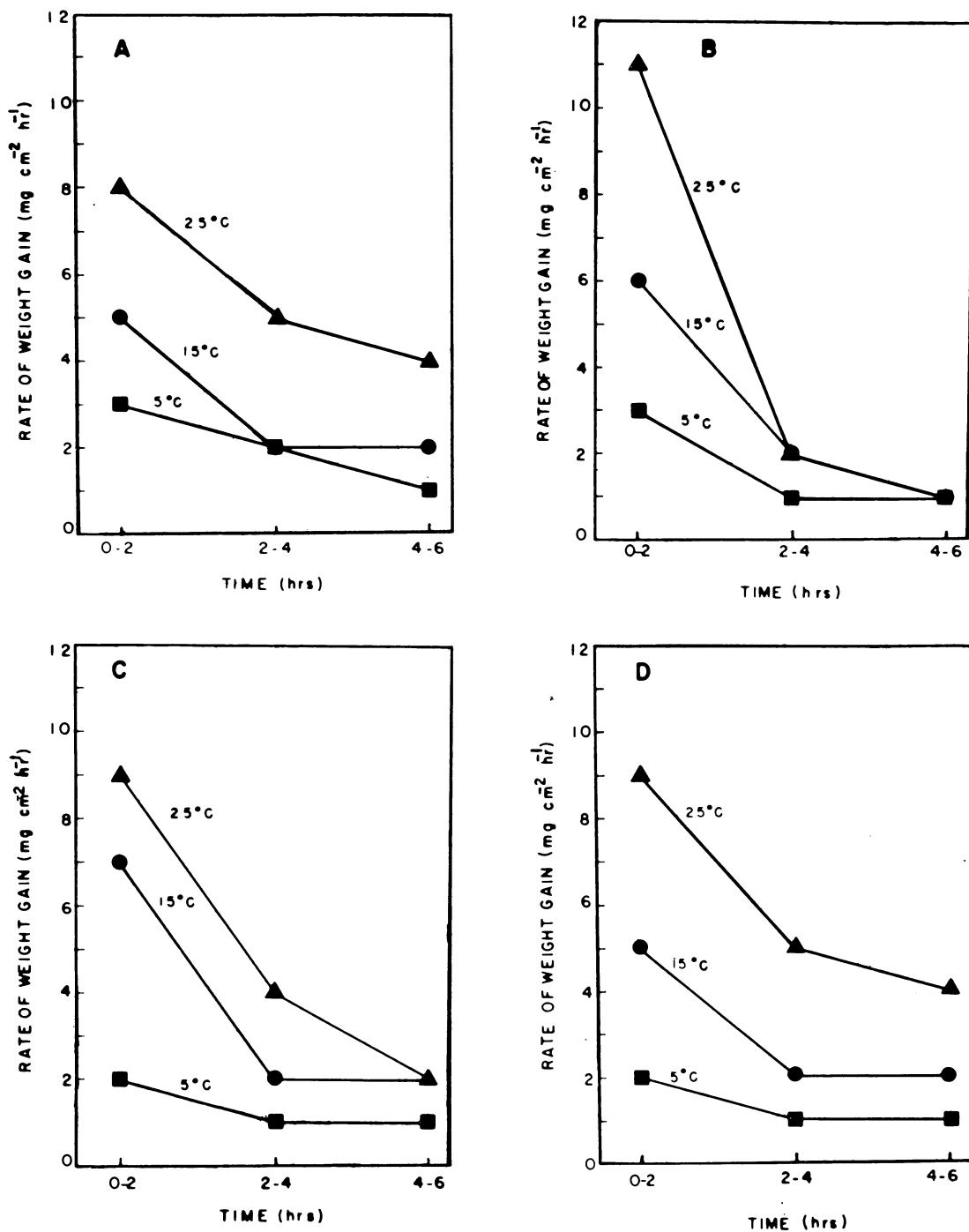


FIG. 12. Effect of time on rate of rehydration in (A) *P. jordani*, (B) *P. ouachitae*, (C) *P. glutinosus* (NC), and (D) *P. glutinosus* (Ozark) acclimated to 5°, 15°, and 25°C.

where μ is the population mean, α_1 is the deviation of the mean of the regression line from μ , β is the slope of the regression line, X is the independent variable, and ϵ is a random variable. In this case X is time period and $X^2 = (\text{time period})^2$. Estimates of β_1 and β_2 were highly significant for all three groups of salamanders. For group I the β_1 estimate

was $-6.38 \text{ mg cm}^{-2} \text{ hr}^{-1}$ (time period) $^{-1}$ with a t -value of -9.04 ($P = 0.000$), and the β_2 estimate was $1.30 \text{ mg cm}^{-2} \text{ hr}^{-1}$ (time period) $^{-2}$ with a t -value of 7.06 ($P = 0.000$; 47 df) (Fig. 12). All four populations exhibited the same pattern. The rate was highest from 0 to 2 hr and lowest from 4 to 6 hr.

The β_1 estimate for group II was -6.54 mg cm^{-2}

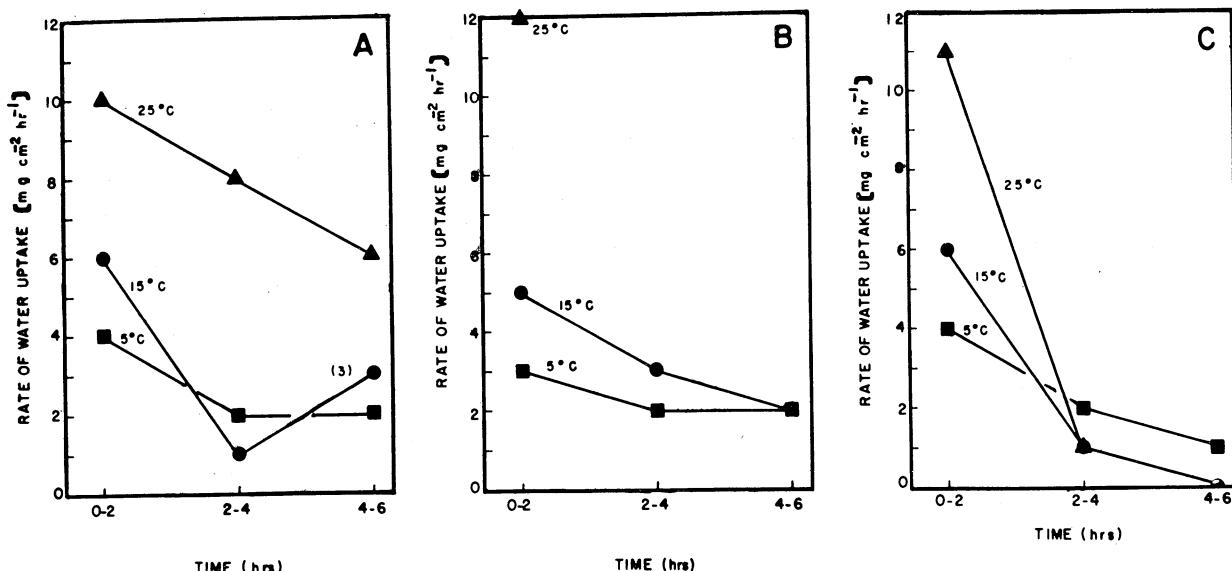


FIG. 13. Effect of time on rate of rehydration of (A) *P. yonahlossee*, (B) *P. caddoensis*, and (C) *E. lucifuga* at 5°, 15°, and 25°C.

hr⁻¹ (time period)⁻¹ with a *t*-value of -4.58 (*P* = 0.000), and the β_2 estimate was 1.25 mg cm⁻² hr⁻¹ (time period)⁻² with a *t*-value of 3.34 (*P* ≤ 0.001; 8 df) (Fig. 13). The effect of time is similar to that shown for the species in group I (Fig. 12). At 25°C *P. caddoensis* completed rehydration in less than 2 hr. The increase in rehydration rate of *P. yonahlossee* at 4–6 hr at 15°C was probably an artifact of the small sample size. In group III (Fig. 14) the β_1 estimate was -4.49 mg cm⁻² hr⁻¹ (time period)⁻¹ with a *t*-value of -5.87 (*P* = 0.000), and the β_2 estimate was 7.90 mg cm⁻² hr⁻¹ (time period)⁻² with a *t*-value of 4.06 (*P* = 0.000; 12 df). As in the other species, rehydration was rapid during the first 2 hr in water and continued at a slower rate for the remaining 4 hr. The increase in rehydration rate of *P. longicrus* at 4–6 hr was probably an artifact of the small sample size.

Temperature had a significant effect on the total rate of rehydration (average for hours 0–6) of the animals in group I (*P* ≤ 0.05). Means for all individuals at 5°, 15°, and 25°C were 2 mg cm⁻² hr⁻¹, 4 mg cm⁻² hr⁻¹, and 8 mg cm⁻² hr⁻¹ respectively. The temperature effect was most evident for *P. ouachitae* and *P. glutinosus* (Ozark) and least apparent for *P. jordani* (Fig. 15). The effect of temperature on the rate during the first 2 hr was most apparent in *P. ouachitae* and least evident in *P. jordani* (Fig. 12). Temperature had a highly significant effect (*P* ≤ 0.01) on the total rate of rehydration (average for hours 0–6) of the salamanders in group II. Rehydration rates were similar at 5° and 15°C but greatly increased at 25°C (Fig. 16).

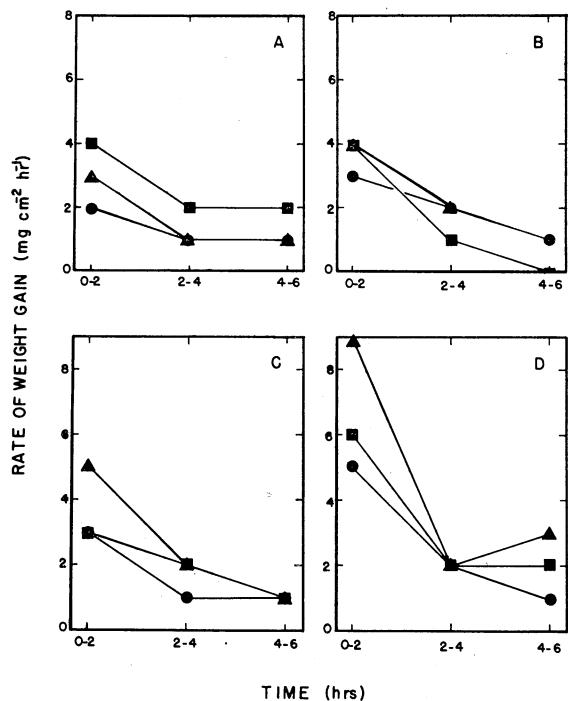


FIG. 14. The effect of time on the rehydration rates of 13 populations of salamanders acclimated to 15°C (LD 16:8).

- (A) *D. monticola* (●—●); *D. ochrophaeus* (NC) (▲—▲); and *D. ochrophaeus* (SM) (■—■).
- (B) *D. fuscus* (●—●); *E. longicauda* (■—■); and *E. multiplicata* (▲—▲).
- (C) *P. c. serratus* (■—■); *P. dorsalis* (●—●); and *P. c. cinereus* (▲—▲).
- (D) *P. longicrus* (▲—▲); *P. jordani* (SM) (■—■); and *P. glutinosus* (Ouachita) (●—●).

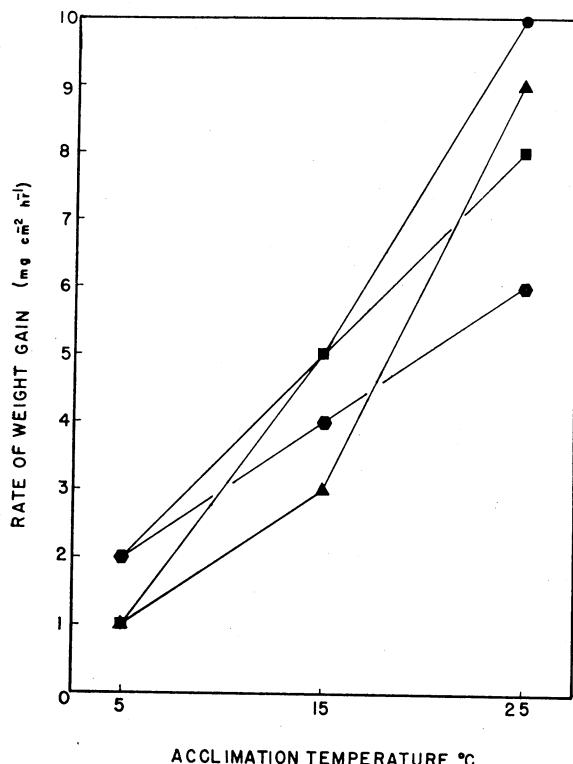


FIG. 15. Rates of rehydration of *P. jordani*, *P. ouachitae*, *P. glutinosus* (Ozark), and *P. glutinosus* (NC) acclimated to three temperatures and two photoperiods. The results for the two light regimes at each temperature are pooled into one sample. *P. jordani* (●—●); *P. ouachitae* (○—○); *P. glutinosus* (Ozark) (▲—▲); *P. glutinosus* (NC) (■—■).

Species differences.—The difference in the rate of rehydration (average for hours 0–6) was highly significant ($P \leq 0.01$) between the 13 populations acclimated to 15°C (Table 6). *Plethodon longicrus* rehydrated most rapidly and differed significantly from other species. The rehydration rate of *E. multiplicata* was not significantly different from those of *E. longicauda*, *P. c. serratus*, *P. dorsalis*, and *P. glutinosus* (Ouachitae). Species and population differences were most evident during the first 2 hr of rehydration (Fig. 14).

Discussion

Dehydration deficit.—Dehydration deficit (percentage weight loss) had a direct effect on the rehydration rates of salamanders. As percentage weight loss due to dehydration increased, rate of water uptake also increased. Spight (1967b) noted a similar effect for some ambystomid and plethodontid salamanders. As percentage dehydration rises and body fluids become more concentrated, water is absorbed through the skin at a correspondingly faster rate.

Large quantities of water were absorbed during the first 2 hr in water, but rehydration rate declined

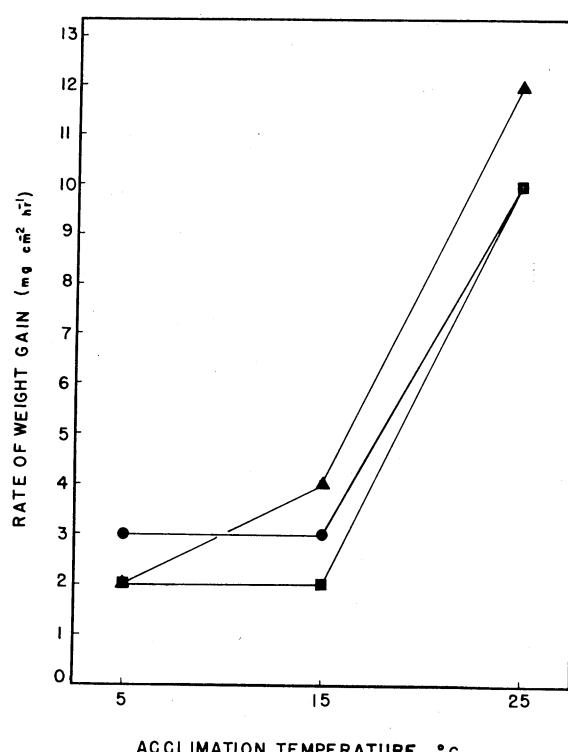


FIG. 16. Rates of rehydration of *P. yonahlossee*, *P. caddoensis*, and *E. lucifuga* acclimated to 5°, 15°, and 25°C (LD 16:8). *P. yonahlossee* (●—●); *P. caddoensis* (▲—▲); *E. lucifuga* (■—■).

during the remaining 4 hr. In some cases weight actually decreased at the fourth and sixth hour. Warburg (1965b, 1967) reported a similar decrease in the rehydration rates of frogs with time. This response was probably caused by a decrease in the osmotic concentration of the body fluids as the total amount of water absorbed increased as the time spent in rehydration increased.

Species comparisons.—Species differences in rehydration rates (averaged for hours 0–6) were significant only in the third series of experiments (group III). Terrestrial species rehydrated more rapidly than aquatic salamanders. The low rates of *P. c. serratus* and *P. dorsalis* were due to their behavior during rehydration. These two species avoided the water and continually climbed up the sides of culture dishes despite repeated efforts to dislodge them. This behavior resulted in a false estimate of their rehydration rates, and individuals often did not gain and sometimes lost weight between weighings. No other salamanders evidenced as great a negative reaction to water. Other plethodonts demonstrated some avoidance of water, but aquatic species tended to remain submerged at all times. The rapid rate of rehydration in large plethodonts of groups I and II may have been due to their inability to climb completely out of the water. A few small individuals climbed up the sides

TABLE 6. Mean rate of rehydration of 13 populations of salamanders acclimated to 15°C (LD 16:8) (group III)

Population	Number of observations	Mean rate ^a (mg cm ⁻² hr ⁻¹)
<i>D. monticola</i>	11	2
<i>P. c. serratus</i>	15	2
<i>P. dorsalis</i>	13	2
<i>D. fuscus</i> (AP)	10	2
<i>D. fuscus</i> (RM)	10	2
<i>D. ochrophaeus</i> (NC)	13	2
<i>E. longicauda</i>	12	3
<i>D. ochrophaeus</i> (SM)	7	3
<i>E. multiplicata</i>	6	3
<i>P. glutinosus</i> (Ouachita)	15	3
<i>P. c. cinereus</i>	10	4
<i>P. jordani</i>	10	5
<i>P. longicrus</i>	6	7

^aAny two means not encompassed by the same vertical line are significantly different as determined by Duncan's new multiple range test.

of dishes for short periods of time and thus rate of rehydration was reduced.

Thorson (1955) and Claussen (1969) found that although various anuran species had significantly different rehydration rates the differences did not necessarily represent differences in the degree of adaptations to terrestrial environments. Warburg (1965b) and Bentley et al. (1958) found that the rate of hydration was more rapid in terrestrial frogs than in more aquatic species. Cohen (1952), Hutchinson (1958), and Spight (1967b) found that the same was true in salamander comparisons, but Gehlbach et al. (1969) were unable to demonstrate such a correlation in *Ambystoma tigrinum* (Green).

Temperature.—Temperature had a direct effect on the rehydration rate with a Q_{10} of 2 in the first series of experiments and 1.8 in the second series. These values indicate that water uptake is due not only to a simple diffusion process (Q_{10} usually 1.3–1.4) but also to changes in the viscosity of water and cell membranes (Giese 1962:202). Increased skin permeability and increased metabolic rates of salamanders at higher temperatures may be responsible for the rapid uptake of water at 25°C. Interspecific differences in rate are probably caused by differences in skin permeability similar to those already demonstrated for anuran species (Schmid and Barden 1965).

In most instances dehydration rates were greater than rehydration rates. Cohen (1952) reported that the rate of dehydration in salamanders was faster than the rate of rehydration, but Gehlbach et al. (1969) found that the opposite was true for *A. tigrinum*. Differences in experimental methods used in these studies may be responsible for conflicting results.

EXCHANGE OF WATER WITH THE SOIL

Amphibians can absorb water from the soil if the

osmotic pressure of their body fluids exceeds that of the soil-moisture tension. Heatwole and Lim (1961) studied water exchange with the soil in *P. cinereus* and defined the "absorption threshold" as "the level of substrate moisture above which there is a net gain in body water by dehydrated salamanders and below which there is a net loss." Spight (1967a) reported that the rate of water exchange between salamanders and the soil is a function of the moisture tension of the soil. Rubial, Trevis, and Roig (1969) and Walker and Whitford (1970) have shown that water uptake from soil is an important factor in the ecology of frogs and toads.

These experiments were conducted to determine the absorption threshold, rate of water exchange at various soil-moisture tensions, and effect of temperature on the rate of water exchange with soil in plethodontid salamanders.

Methods

Salamanders were dehydrated over glycerol-water solutions for 4 hr, weighed, and placed into new 1-pint jars containing about 50 g of soil of various moisture contents. Salamanders were covered with soil, and a piece of screen wire was inserted into the jar to prevent them from climbing the sides. Jars were sealed and returned to the environmental chamber at the appropriate acclimation condition. After 4 hr (2 hr for very small individuals) salamanders were removed and a soil sample taken. Each animal was rinsed in tap water, blotted dry in paper towels, and weighed. Results were expressed as weight change (surface area)⁻¹ time⁻¹ in mg cm⁻² (4 hr)⁻¹. All tests were run between 1140 and 2230 CST. Soil samples were dried for 12 hr at 125°C (constant weight), and soil moisture was calculated from the change in weight.

The experimental soil was a homogeneous mixture of three soils taken from the Ozarks, from Camp Albert Pike, and from Grandfather Mountain, N.C. Soil moisture ranged from 9% to 55% of dry weight. Soil-moisture tensions up to 1 atm were determined with a ceramic plate apparatus (Richards and Fireman 1943), and those above 1 atm were determined with a pressure membrane extraction apparatus (Richards 1941, 1947, 1949). Samples were saturated and Fig. 17 represents the drying phase of the hysteresis curve for this soil.

Results

Absorption threshold.—The rate of water exchange with the soil was directly related to the percentage of soil moisture. The species had similar absorption thresholds (1.2–2.8 atm) and absorbed water from unsaturated soil at rates that were dependent upon the water content of the soil (Fig. 18–19). These results confirm those of Spight (1967a)

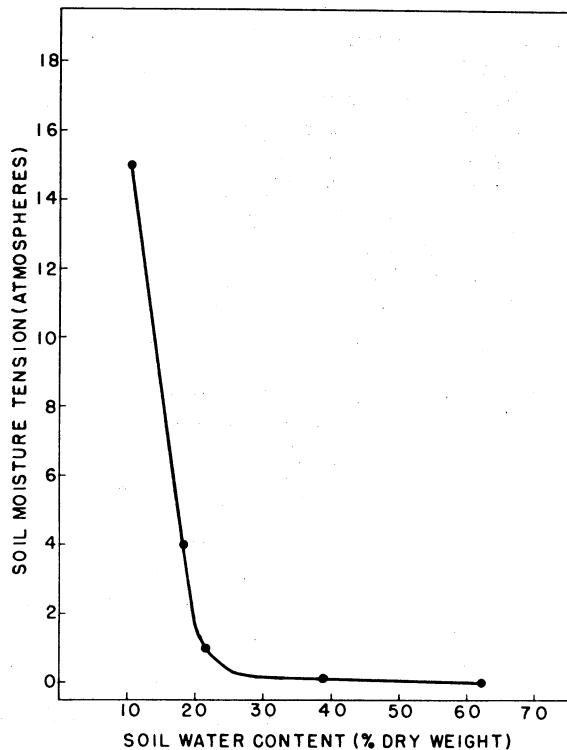


FIG. 17. Characteristic curve of soil-moisture tension of the experimental soil. Each point represents the mean of two values.

for six species of salamanders from North Carolina. These salamanders had an absorption threshold of 2 atm and exchanged water with soil at similar rates in unsaturated soil. Heatwole and Lim (1961) reported that *P. cinereus* tested at room temperature had an absorption threshold of 1–1.5 atm.

Results of the experiments conducted at 5° and 15°C are not presented since the slow absorption rates of salamanders at these temperatures underestimated the ability of salamanders to take up water from soil. At 25°C the linear regressions generated by the ANCOVA were an inaccurate estimate of the absorption threshold, over-estimating the ability of salamanders to absorb water from soils at high soil-moisture tensions. Another ANCOVA indicated that the regression for water exchange was not quadratic. Therefore the results were plotted and a regression line drawn by sight to best fit the data points.

Discussion

Ecological consequences.—Soils normally have a moisture tension of 0.33 atm after gravitational water has drained away. Since this occurs within 3 days after a heavy rain (Oosting 1956:179) salamanders normally have an operating range of soil-moisture tensions of 0.33–2.8 atm. This compares to a range of 0.33–2.7 atm reported for frogs by Walker and

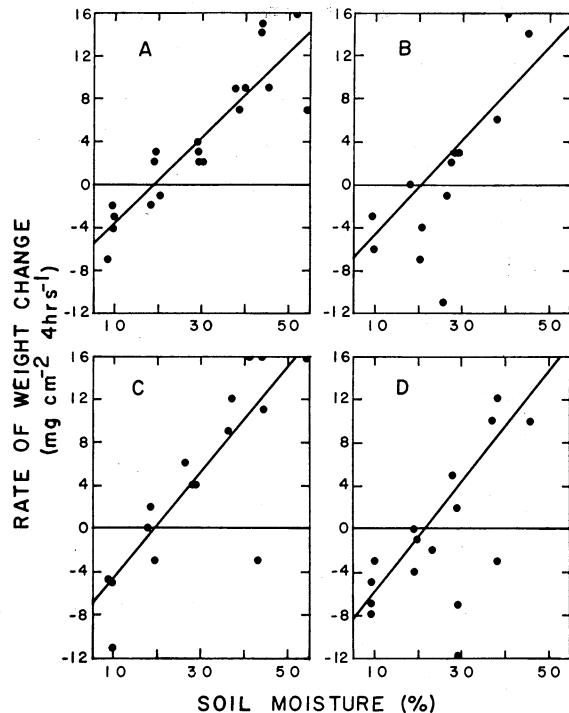


FIG. 18. The rate of weight change of (A) *P. jordani*, (B) *P. ouachitae*, (C) *P. glutinosus* (Ozark), and (D) *P. glutinosus* (NC) rehydrated in moist soil at 25°C.

Whitford (1970) and a range of 0.33–15 atm reported for desert toads (Ruibal et al. 1969). If a salamander is in soil with a tension greater than 2.8 atm it will lose water to the soil. As this water is absorbed by the soil a "wetting front" (Spight 1967a) will be formed between the wet region of soil adjacent to the salamander and the drier region nearby. The characteristics of this front are such that there will be little or no movement of water from the wet to the dry region (Bodman and Colman 1944). Thus the salamander will be surrounded by soil with a low soil-moisture tension and will either lose water at a reduced rate because of a reduction in the steepness of the salamander-soil moisture gradient or will stop losing water altogether because of an equilibrium between the osmotic pressure of its body fluids and the moisture tension of the soil.

Soil factors.—Exchange of water with the soil is dependent upon several factors related to the composition of the soil. Size of soil particles and their compactness influence the quantity and movement of capillary water and the amount of water vapor between the particles. Organic content of the soil will alter its water-holding capacity, and mineral content will influence the ionic makeup of the soil water. Orientation of the soil particles in relation to the skin of the salamander will affect the availability of capillary water to the animal. Whenever the salamander moves and changes the orientation of the nearby soil

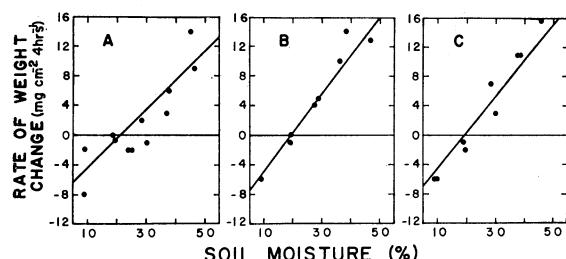


FIG. 19. Rate of weight change of (A) *P. yonahlossee*, (B) *P. caddoensis*, and (C) *E. lucifuga* rehydrated in moist soil at 25°C.

particles, it will alter the relationship and inhibit the formation of a wetting front.

Percentage of moisture in the soil is not a good indicator of the availability of soil water to the salamander since the same soil-moisture tension in two soils may represent very different water contents. The moisture-tension relationship remains constant from soil to soil and permits prediction of salamander-water relationships in different soils (Spirge 1967a). Thus, if a salamander can take up water from one soil at a soil-moisture tension of 1 atm, it should be able to absorb water from other soils at 1 atm, even if their moisture-tension curves are different. The use of percentage of water in the soil as a comparison of the water availability in different salamander habitats has little predictive value unless a determination is made of the moisture-tension curve of each soil.

PREFERRED RELATIVE HUMIDITY

Terrestrial plethodontid salamanders are active at night when the relative humidity is high. On dry nights they remain close to their refuges, often with only their heads visible. They appear to detect the amount of water vapor in the air and alter their behavior patterns accordingly. The purpose of this portion of the study was to determine if the study species were sensitive to the moisture content of the air, and, if so, whether there were interspecific differences in their responses. Observations were made with a relative humidity chamber in which salamanders were exposed to a range of VPD from 1.0 to 22.5 mm Hg.

Methods

Gradient design.—A linear humidity gradient was established in a wooden box, internal dimensions 203.2 by 10.2 by 10.2 cm. The box was subdivided into an upper selection chamber, 203.2 by 10.2 by 5.1 cm, and 10 lower sections 19.1 by 8.9 by 5.1 cm, divided by 1.3-cm partitions. A continuous length of screen wire separated the upper and lower halves of the box and provided a uniform floor for the selection chamber. Removable wooden boxes were in-

TABLE 7. Specific gravity, expected and actual relative humidity (RH), and vapor pressure deficit (VPD) of glycerol-water solutions used to maintain the humidity gradient

Sector	Specific gravity	Expected RH ^a (%)	Actual RH ^b (%)	Vapor pressure deficit (mm Hg)
1	CaSO ₄	0	18.9 (6.8–36.5)	15.8 (12.4–18.2)
2	1.261°	0	38.2 (16.8–52.5)	12.0 (9.2–16.2)
3	1.250	20.0	46.9 (27.6–58.7)	10.3 (8.0–14.1)
4	1.240	30.0	52.1 (44.5–66.5)	9.3 (6.5–13.9)
5	1.216	47.0	55.9 (47.5–67.0)	8.6 (6.4–10.2)
6	1.178	66.0	60.8 (52.0–73.0)	7.6 (5.3–9.4)
7	1.155	74.0	68.0 (56.5–83.0)	6.2 (3.3–8.5)
8	1.137	80.0	72.4 (61.0–85.5)	5.4 (2.8–7.6)
9	1.068	92.0	83.4 (72.0–92.5)	3.2 (1.5–5.4)
10	1.000	100.0	90.3 (82.5–96.0)	1.9 (0.8–3.4)

^aExpected RH was computed with data from Braun and Braun (1958).

^bEach actual RH value is the mean of 68 observations.

°Pure glycerol.

serted into the bottom sections through access doors on the side of the box. Ten evenly spaced 2-cm holes centered in the wall of the selection chamber above the doors served as access ports for the RH sensors. Plexiglas partitions (10.2 by 5.1 by 0.3 cm) divided the selection chamber into 10 sectors corresponding to the lower sections and helped to maintain the humidity gradient. Semicircular holes (radius 1 inch) in these partitions allowed salamanders to pass freely between sectors.

A humidity gradient was established by putting anhydrous CaSO₄ in the box of one end section, distilled water in the box of the other end section, and glycerol-water solutions in those in between. The specific gravity, expected RH, actual RH, and VPD of these solutions are presented in Table 7. A satisfactory gradient was usually established within 4 hr and remained stable for several days. Fresh solutions were prepared for each acclimation condition.

Relative humidity was monitored with an electric hygrometer indicator (Hydrodynamics, Inc., Model 15-3001) and narrow range sensing elements. This system has an accuracy of $\pm 1.5\%$ RH. Temperature was measured with a standardized mercury thermometer graduated to 1.0°C.

The gradient box was maintained in a laboratory with a temperature of 18–26°C. Room temperature did not fluctuate more than 2.5°C daily. There was a very slight temperature gradient in the selection chamber with a maximum range of 1.4°C from the cool to the warm end (measured with YSI telemeter and probes). Diffuse light was provided by overhead fluorescent lamps during the light portion of the experimental photoperiods. During the dark phase the top of the gradient was covered with a double layer of black cloth.

Testing procedure.—Groups of up to 10 small or five large (> 5.00 g) individuals were placed into the selection chamber, one per sector. After approx-

imately 2 hr the RH of each sector and temperature of the gradient were recorded and the positions of the salamanders noted. Preliminary experiments demonstrated that the positions of salamanders acclimated to 15°C stabilized in less than 2 hr. Longer test periods were avoided to prevent the possibility of excess dehydration at 15° and 25°C. Tests were conducted between 0610 and 2230 CST.

In preliminary experiments animals placed in the selection chamber with water in all the lower sections showed a slight tendency to go to the end sectors. To counteract this, each animal was tested twice, whenever possible, the gradient being reversed before the second trial.

Results

Salamander behavior.—Salamanders put into the selection chamber actively explored it for approximately 1 hr. They moved slowly from sector to sector sometimes stopping for a few minutes before entering the next area. Usually they spent only a very short time in the sectors over CaSO_4 and glycerol. Finally their position stabilized in those sectors where the VPD was low. Some animals stayed next to the walls when they moved and stopped when they came to the plexiglas partitions. This cornering effect hindered the movement of a few individuals, but did not alter the behavior of the remainder of the salamanders. I did not observe any change in selection of individuals because of grouping of salamanders in one or more sectors.

Another type of behavior was apparent in some animals. These salamanders explored the gradient until they entered a sector with a high VPD, particularly the end one over CaSO_4 . Here they stopped and curled up into a water-retention position where they remained until removed at the end of the test period. This type of activity was especially noticeable when animals were tested a second time. Often they would go immediately to the end sector that had the highest vapor pressure during the initial test. Since the gradient was reversed this sector now had the lowest vapor pressure. Salamanders that did not immediately retreat usually took up water-retention positions, groups of salamanders often forming a spherical mass in one of the corners. These salamanders did not clump together until they had already selected a particular sector. Then they congregated in a mass, possibly in response to the high VPD in the low RH sectors.

Large salamanders (>7.0 g) did not respond to the gradient as well as other individuals. Large quantities of water vapor evaporated from their skin often created high vapor pressures in the low RH sectors so that they remained in these areas and did not explore the rest of the gradient.

Despite these difficulties most salamanders re-

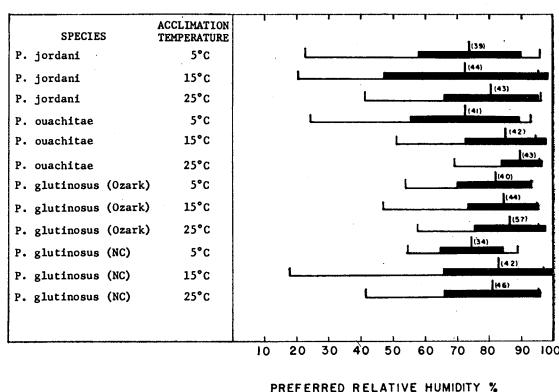
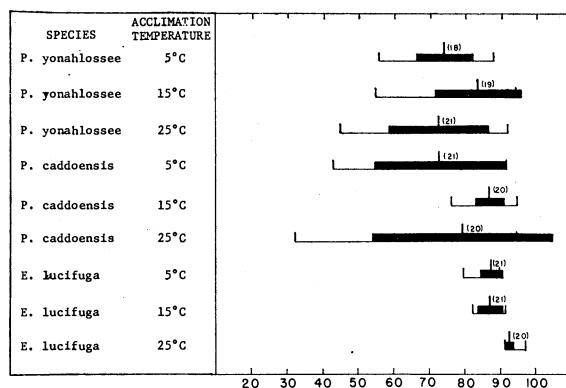


FIG. 20. Preferred relative humidity of *P. jordani*, *P. ouachitae*, *P. glutinosus* (Ozark), and *P. glutinosus* (NC) acclimated to 5°, 15°, and 25°C (two light regimes at each temperature are pooled into one sample). Means are represented by long vertical lines. Horizontal lines bounded by short vertical lines represent the range. Black rectangles represent ± 1 standard deviation (SD). Number of observations in parentheses.

sponded to differences in vapor pressure in the gradient and remained in sectors with a high RH.

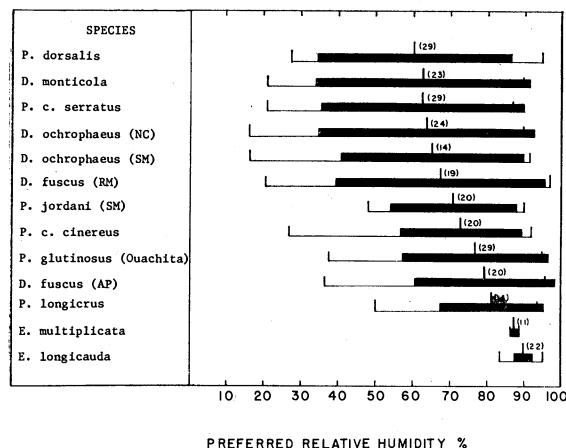
Preferred relative humidity.—Salamanders clearly selected areas of high relative humidity in the gradient (Fig. 20, 21, 22). Actual success in doing so is not shown by these graphs because during many tests the highest RH in the gradient was as low as 80–85%. Results from such tests lowered the average values even though the salamanders may have selected the highest available RH in the gradient. Therefore the percentage success of each individual was computed by dividing the preferred RH by the highest RH available in the gradient for that test period and multiplying by 100. In many instances this relative success was above 90%. These adjusted values of relative success (Table 8) give a better indication of the salamanders' ability to select the highest possible RH available in the humidity gradient.

Species differences in all three test groups were highly significant ($P \leq 0.01$). Population differences for the salamanders in group III are shown in Table 9. Ranges and standard deviation for most of the salamanders in group III (Fig. 22) were large, indicating less precise selection than that seen in the other two groups (Fig. 20 and 21). Members of the genus *Eurycea* showed the most precise RH selection and the highest percentage success. Members of the genus *Desmognathus* along with *P. dorsalis* and *P. c. serratus* had the least success in the gradient. In the first test group (all six acclimation states) relative success increased as the acclimation temperature increased (Table 8). In group II *E. lucifuga* had the greatest success at all three temperatures (Table 8). *Eurycea longicauda* and *E. multiplicata* (Table 8) were the most successful species in group III.



PREFERRED RELATIVE HUMIDITY %

FIG. 21. Preferred relative humidity of *P. yonahlossee*, *P. caddoensis*, and *E. lucifuga* acclimated to 5°, 15°, and 25°C (LD 16:8). See Fig. 20 for explanation of symbols.



PREFERRED RELATIVE HUMIDITY %

FIG. 22. Preferred relative humidity of 13 populations of salamanders acclimated to 15°C (LD 16:8). See Fig. 20 for explanation of symbols.

Time of day had a significant effect on the preferred RH of the animals in group I. The β estimate was $-0.464\% \text{ hr}^{-1}$ with a t -value of -2.15 ($P \leq 0.032$; 47 df). Preferred RH decreased as the time of day advanced at the rate of $0.46\% \text{ hr}^{-1}$. Differences due to photoperiod were not significant in these tests.

Discussion

Species comparisons.—Salamanders in the humidity gradient responded to differences in the moisture content of the air and remained in those sectors with a high RH. Interspecific differences were apparent, but were not always correlated with habitat preferences. The three species of *Eurycea* selected the highest RH and had the highest success of all the salamanders tested. Of these three species *E. multiplicata* is primarily aquatic, *E. lucifuga* is an inhabitant of moist ledges and caves, and *E. longicauda* is more

TABLE 8. Relative success of 20 populations of salamanders in selecting the highest possible relative humidity available in the relative humidity gradient—expressed as percentage^a (each value is the mean of all values calculated for that population at each acclimation temperature)

Population	Acclimation temperature (°C)		
	5	15	25
<i>P. jordani</i>	80.6 ^b	79.7 ^b	88.4 ^b
<i>P. ouachitae</i>	80.9	93.4	97.5
<i>P. glutinosus</i> (Ozark)	90.0	92.0	94.0
<i>P. glutinosus</i> (NC)	82.2	89.4	88.4
Group II			
<i>P. yonahlossee</i>	81.5	91.6	79.4
<i>P. caddoensis</i>	81.2	98.3	84.6
<i>E. lucifuga</i>	98.2	100.0	100.0
Group III			
<i>P. dorsalis</i>	—	67.4	—
<i>D. monticola</i>	—	72.4	—
<i>P. c. serratus</i>	—	72.5	—
<i>D. ochrophaeus</i> (NC)	—	72.0	—
<i>D. ochrophaeus</i> (SM)	—	72.6	—
<i>D. fuscus</i> (RM)	—	76.7	—
<i>P. jordani</i> (SM)	—	79.5	—
<i>P. c. cinereus</i>	—	81.4	—
<i>P. glutinosus</i> (Ouachita)	—	85.4	—
<i>D. fuscus</i> (AP)	—	85.6	—
<i>P. longicrus</i>	—	91.6	—
<i>E. multiplicata</i>	—	100.0	—
<i>E. longicauda</i>	—	99.6	—

^aPercentage success was computed for each individual in each test by dividing the preferred relative humidity by the highest relative humidity available in the gradient for that test period and multiplying by 100.

^bResults for the two photoperiods at each temperature are pooled into one sample.

TABLE 9. Mean preferred relative humidity of 13 populations of salamanders acclimated to 15°C (LD 16:8) (group III)

Population	Number of observations	Preferred RH (%) ^a
<i>P. dorsalis</i>	29	60.4
<i>D. monticola</i>	23	62.8
<i>P. c. serratus</i>	29	62.8
<i>D. ochrophaeus</i> (NC)	24	63.7
<i>D. ochrophaeus</i> (SM)	14	65.3
<i>D. fuscus</i> (RM)	19	67.5
<i>P. jordani</i> (SM)	20	70.1
<i>P. c. cinereus</i>	20	73.0
<i>P. glutinosus</i> (Ouachita)	29	76.9
<i>D. fuscus</i> (AP)	20	79.6
<i>P. longicrus</i>	14	81.4
<i>E. multiplicata</i>	11	87.7
<i>E. longicauda</i>	22	90.0

^aAny two means not encompassed by the same vertical line are significantly different as determined by Duncan's new multiple range test.

terrestrial and less intimately associated with caves and ledges than *E. lucifuga*. In general, members of the terrestrial genus *Plethodon* had a greater success than members of the more aquatic genus *Desmognathus*. *Desmognathus fuscus* (AP) was collected from a temporary stream and had a success similar

TABLE 10. Temperatures and relative humidities at the capture sites of salamanders—Arkansas, 1968, 1969

Species	Number of collections	Number of animals	Temperature (°C)		Relative humidity (%)	
			Range	Mean	Range	Mean
<i>P. ouachitae</i>	4	41	15.2–21.4	17.0	88.0–100.0	99.2
<i>P. caddoensis</i>	9	39	11.6–22.8	16.2	83.0–100.0	99.0
<i>P. c. serratus</i>	2	3	15.3–15.8	15.6	—	100.0
<i>P. glutinosus</i> (Ouachita)	2	2	14.4–15.8	15.1	—	100.0
<i>P. glutinosus</i> (Ozark)	12	33	12.2–19.5	15.2	94.0–100.0	99.3
<i>P. dorsalis</i>	1	8	11.4–13.0	12.0	97.0–100.0	99.6
<i>E. lucifuga</i>	6	11	13.5–22.2	15.6	95.0–100.0	99.5
<i>E. longicauda</i>	3	3	15.0–22.2	17.5	95.0–100.0	98.3

to that of several plethodonts. *Plethodon dorsalis* and *P. c. serratus* had the lowest success of all the salamanders tested at 15°C and avoided air with a high moisture content. These two species are inhabitants of dry microhabitats.

Several workers have studied the reactions of animals to atmospheric moisture. Shelford (1913, 1914) found that *P. glutinosus* was more sensitive to dryness than *P. cinereus* and that both species turned away from a stream of dry air. Heatwole (1962) reported that *P. cinereus* moved to the higher relative humidities available in a gradient and that this response was more pronounced after desiccation. Platt, Collins, and Witherspoon (1957) reported that the optimum RH of the mosquito *Anopheles quadrimaculatus* Say in a moisture gradient was between 70% and 80% and that higher and lower humidities were sharply avoided. Reichle (1967) found that bog pselaphid beetles selected the highest humidity available in a gradient. Gunn and Cosway (1938) reported that the humidity reaction of cockroaches, *Blatta orientalis* L., in a linear gradient was variable and not intense. Gunn (1937) found that the woodlouse *Porcellio scaber* (Latreille) selected high humidities in a gradient, and Kennedy (1937) reported that the locust *Locusta migratoria migratorioides* R. and F., tested in an alternative chamber apparatus, demonstrated a preference for dry air in all parts of the humidity range. Apparently animals normally found in habitats where the air is saturated with moisture tend to select the highest available RH in a gradient, whereas species from drier habitats prefer low or intermediate RH levels.

Adaptive value of the RH response and a possible mechanism.—Selection of high humidities by salamanders is of adaptive value because of their moist skin and high rate of evaporative water loss. Their ability to distinguish between air of high and low VPD and to restrict their activity periods to times of high atmospheric humidity enables them to occupy terrestrial habitats and avoid excessive dehydration stress during normal activities.

The mechanism by which salamanders distinguish differences in the moisture content of the atmosphere is unknown. Hygroreceptors have been demonstrated

in several families of Coleoptera (Roth and Willis 1951), and a water taste has been reported for frogs (Zotterman 1950), but no evidence of hygroreceptors has been reported in salamanders. The response of salamanders to dry air may be due to a concentration of body fluids caused by dehydration. Partially desiccated *P. cinereus* demonstrated a more positive response in a humidity gradient than hydrated individuals (Heatwole 1962). Decreased success of the salamanders acclimated to 5°C may have been due to a reduction in their rate of water loss while they were warming to room temperature. Since their body fluids were less concentrated than those of the salamanders at 15° and 25°C, they did not respond as well to the moisture content of the air in the gradient. If these salamanders had been left in the gradient long enough to cause a large increase in their total water loss, they may have shown a corresponding increase in their success.

FIELD STUDIES

An attempt has been made to correlate ecological observations made in the field while collecting salamanders with the results of laboratory studies. Microclimate temperatures and relative humidities were recorded, and observations were made on the microhabitat, behavior, and general ecology of the species collected. Four temperatures were measured: air temperature, 4 ft above the ground; ground temperature, thermistor bead just under the surface; ground air temperature, 4 inches above the ground; and soil temperature, 4 inches below the surface. These were recorded with YSI telethermometers and thermistor probes. Relative humidities and air temperatures of salamander microenvironment were recorded with a thermistor psychrometer (Atkins Technical, Inc.) at the sites where animals were captured. Weather conditions and collection times were also recorded.

Arkansas salamanders

Temperatures and relative humidities of salamander microenvironments were measured with the thermistor psychrometer just above the substrate at the exact place where the salamander was captured

TABLE 11. Microhabitat temperatures of *P. ouachitae*, *P. glutinosus*, and *P. c. serratus* collected on Rich Mountain, Arkansas, in 1968 and 1969

Location and date	Time	Temperature (°C)				Number of salamanders and species
		Air 4 ft	Ground air	Ground	Soil	
North slope						
3/18/68	11:15	16.5	16.0	14.8	10.0	2 (<i>P. glutinosus</i>)
3/18/68	12:00	16.0	16.0	14.0	11.8	3 (<i>P. ouachitae</i>)
Fire tower						
3/18/68	15:20	14.0	14.0	13.0	10.0	1 (<i>P. ouachitae</i>)
3/18/68	16:15	17.0	15.0	14.0	11.8	2 (<i>P. ouachitae</i>)
North slope						
3/19/68	9:50	16.0	16.0	14.0	12.5	5 (2 <i>P. ouachitae</i> , 3 <i>P. glutinosus</i>)
3/20/68	10:50	9.5	8.0	7.5	10.0	5 (3 <i>P. ouachitae</i> , 2 <i>P. glutinosus</i>)
3/29/68	11:30	26.0	26.0	22.0	17.0	1 (<i>P. glutinosus</i>)
3/31/68	10:45	16.9	16.5	15.1	14.6	3 (2 <i>P. ouachitae</i> , 1 <i>P. glutinosus</i>)
Fire tower						
4/18/68	20:00	18.0	17.2	17.5	17.0	71 (<i>P. ouachitae</i>)
9/24/68	22:00		15.6			47 (45 <i>P. ouachitae</i> , 2 <i>P. glutinosus</i>)
4/8/69	22:30	16.0	16.0	14.0		33 (28 <i>P. ouachitae</i> , 2 <i>P. glutinosus</i> , 3 <i>P. c. serratus</i>)
North slope						
4/9/69	11:00	28.3	29.1	20.5	17.0	1 (<i>P. glutinosus</i>)
Fire tower						
10/11/69	22:30		20.0			6 (5 <i>P. ouachitae</i> , 1 <i>P. c. serratus</i>)

TABLE 12. Microhabitat temperatures of *P. caddoensis*, *P. glutinosus*, *P. c. serratus*, and *D. fuscus* collected at Camp Albert Pike, Arkansas, in 1968 and 1969

Date	Time	Temperature (°C)				Number of salamanders and species
		Air 4 ft	Ground air	Ground	Soil	
3/29/68	21:45	14.4	14.4			2 (1 <i>P. caddoensis</i> , 1 <i>P. glutinosus</i>)
3/30/68	12:00	18.5	18.0	16.5	14.0	3 (2 <i>P. caddoensis</i> , 1 <i>P. c. serratus</i>)
3/30/68	14:00	17.9	17.5	16.8	14.8	8 (<i>D. fuscus</i>)
3/30/68	14:30	20.1	19.5	18.7	13.8	1 (<i>P. caddoensis</i>)
3/30/68	15:45	21.0	19.9	18.5	17.9	1 (<i>P. glutinosus</i>)
3/30/68	20:00	16.5	16.5	16.8	14.8	19 (16 <i>P. caddoensis</i> , 3 <i>P. glutinosus</i>)
7/5/68	19:40	16.1	16.0	15.9	15.0	3 (<i>P. caddoensis</i>)
6/17/68	19:20	23.2	23.5	23.0	22.0	1 (<i>P. caddoensis</i>)
4/8/69	20:30		19.4			4 (2 <i>P. caddoensis</i> , 2 <i>P. glutinosus</i>)
4/18/69	21:00	11.6	11.8			7 (6 <i>P. caddoensis</i> , 1 <i>P. glutinosus</i>)
5/18/69 ^a	15:00			16.6 ^b		10 (<i>D. fuscus</i>)
5/18/69	21:30	15.2	16.1	16.2	16.9	33 (26 <i>P. caddoensis</i> , 7 <i>P. c. serratus</i>)
10/11/69	20:45		22.8			37 (35 <i>P. caddoensis</i> , 2 <i>P. glutinosus</i>)

^aStream 1 mile west of Camp Albert Pike.^bIn water.

(Table 10). Arkansas salamanders were active only when the RH was very high. The range of temperatures for *P. dorsalis* is small and the mean is low because all eight individuals were found in the same cave at the same time.

Temperature data for salamanders collected in the Ouachita Mountains are presented in Tables 11 and 12. The majority of the *P. ouachitae* were collected near a rock wall at the base of a fire tower on Rich Mountain. Activity was highest on mild nights (15–19°C) in the spring and fall when the area was covered with fog. *Plethodon glutinosus* was found primarily at the base of the north slope. It was sel-

dom found at the same location as *P. ouachitae* and was always in wetter microhabitats. *Plethodon caddoensis* was abundant on a north-facing slope in Camp Albert Pike. Activity was greatest on mild (ground air temperature 16.1°–22.8°C), partly cloudy nights in the spring and fall when the substrate was still wet from a recent rain.

Plethodon glutinosus, *E. lucifuga*, and *E. longicauda* in the Ozarks were associated with caves, escarpments, and wet ledges. The low microhabitat temperatures recorded for these salamanders (Table 13) were due to the moderating influence of caves and ledges. Temperatures near the mouth of Logan

TABLE 13. Microhabitat temperatures of *P. glutinosus*, *P. dorsalis*, *E. lucifuga*, and *E. longicauda* collected in the Ozark Mountains of Arkansas in 1968 and 1969

Location and date	Time	Temperature (°C)			Soil	Number of salamanders and species
		Air 4 ft.	Ground air	Ground		
Delap caves	4/2/69	18:57	15.8	16.1	13.8	12.4
	4/17/69	20:00		13.3		29 (21 <i>P. glutinosus</i> , 8 <i>E. lucifuga</i>)
	5/5/69	21:00			18.0	56+ (25 <i>P. glutinosus</i> , 30 <i>E. lucifuga</i> , 1 <i>E. longicauda</i>)
Denny cave	5/11/68	21:35	16.0	16.0	16.1	16.7
Granny Dean cave	8/28/68	20:00		13.9		9 (6 <i>P. glutinosus</i> , 2 <i>E. lucifuga</i> , 1 <i>E. longicauda</i>)
	4/22/69	13:20		12.0		10 (2 <i>P. glutinosus</i> , 8 <i>P. dorsalis</i>)
Johnson, Ark.	4/30/69	10:00			12.5	
Logan Springs cave	5/3/68	22:00	15.0	15.0	15.2	15.0
	7/8/68	21:10	18.2	18.9	17.5	17.1
	4/3/69	19:03	19.2	17.2	15.0	13.2
War Eagle cave	5/8/68	21:20	15.9	15.1	15.7	15.0

*Estimated number (not collected).

TABLE 14. Microhabitat temperatures (°C) near the mouth of Logan Springs cave, Arkansas, and in the nearby forest in April and July, 1968

Position	April 3, 1968		July 8, 1968	
	Cave	Forest	Cave	Forest
Air 4 ft	16.5	20.0	18.2	23.2
Ground air			18.9	23.0
Ground	15.0	20.0	17.5	22.3
Soil			17.1	22.0

Springs cave were substantially lower than in the neighboring forest (Table 14).

North Carolina salamanders

Microhabitat temperatures were recorded in North Carolina at the salamander collection sites (Table 15). Low temperatures limited salamander activity. On the nights of May 30 and 31, 1968, ground air temperatures were 11.5° and 12.5°C and substrate temperatures were 11.1° and 12.1°C. On both nights only a few *P. yonahlossee* and *P. glutinosus* were collected. Salamander activity almost ceased within 1 hr after dark. A few animals were seen in sheltered positions with their bodies held close to the substrate.

Temperature and relative humidity

The range of substrate (ground) temperatures recorded for plethodontid salamanders in this study is similar to ranges reported for these and other

species by Taub (1961), Brattstrom (1963), and Thurow (1963).

Relative humidities recorded for Arkansas salamanders at the time of collection indicated that these species were active only when the VPD was low. On several occasions at Camp Albert Pike when it was windy, no salamanders were collected even though the RH was high. The wind apparently stripped away the boundary layer of moist air usually present around the salamander creating unfavorable conditions for salamander activity. On nights when the RH was low at the Old Yonahlossee Road location *P. yonahlossee*, *P. glutinosus*, and *P. jordani* were often seen with only their heads sticking out from under a rock or log. The same was true for *E. lucifuga* and *P. glutinosus* in the Ozarks. These salamanders appeared to respond to the moisture content of the air and altered their activity accordingly. This observation was substantiated by the results of the RH gradient tests which demonstrated that these species tended to select the highest available level of atmospheric humidity in the gradient. It was also consistent with the observations of Hutchinson (1958) who found that moisture was the most important factor affecting the distribution of *E. lucifuga* in the twilight zone of caves.

Ecological observations

Observations on the ecology of *P. ouachitae* and *P. caddoensis* made in this study were generally in

TABLE 15. Microhabitat temperatures of *P. yonahlossee*, *P. jordani*, *P. glutinosus*, *P. c. cinereus*, and *P. longicrus* collected in North Carolina in 1968 and 1969

Location and date	Time	Temperature (°C)				Number of salamanders and species
		Air 4 ft	Ground air	Ground	Soil	
Old Yonahlossee Road						
5/31/68	13:00	16.0	16.5	15.1	12.0	17+ (7 <i>P. yonahlossee</i> , several ^a <i>P. jordani</i> , 10 <i>P. glutinosus</i>)
7/25/68	13:00	26.4	24.7	21.8	—	6 (2 <i>P. jordani</i> , 4 <i>P. glutinosus</i>)
5/29/68	15:00	15.1	14.0	14.0	12.1	14+ (11 <i>P. yonahlossee</i> , several <i>P. jordani</i> , 3 <i>P. glutinosus</i>)
5/28/68	16:00	11.0	11.2	11.9	12.0	15 (2 <i>P. yonahlossee</i> , 10 <i>P. jordani</i> , 3 <i>P. glutinosus</i>)
6/1/68	16:00	19.8	20.0	16.8	13.5	9+ (1 <i>P. yonahlossee</i> , several <i>P. jordani</i> , 8 <i>P. glutinosus</i>)
5/29/68	20:00	11.0	11.1	11.2	11.2	27+ (17 <i>P. yonahlossee</i> , several <i>P. jordani</i> , 10 <i>P. glutinosus</i>)
7/25/68	20:25	20.5	20.7	20.3	18.9	38+ (12 <i>P. yonahlossee</i> , several <i>P. jordani</i> , 26 <i>P. glutinosus</i>)
5/31/68	20:30	13.0	12.5	12.1	12.1	9+ (6 <i>P. yonahlossee</i> , several <i>P. jordani</i> , 3 <i>P. glutinosus</i>)
5/31/69	20:30	14.3	14.1	14.0	12.9	185 (17 <i>P. yonahlossee</i> , 111 <i>P. jordani</i> , 40 <i>P. glutinosus</i> , 17 <i>P. c. cinereus</i>)
7/23/68	20:45	18.5	18.8	18.8	18.0	56 (15 <i>P. yonahlossee</i> , 32 <i>P. jordani</i> , 9 <i>P. glutinosus</i>)
7/24/68	20:45	20.0	19.7	18.9	18.0	91+ (28 <i>P. yonahlossee</i> , 50+ <i>P. jordani</i> , 13 <i>P. glutinosus</i>)
6/1/69	20:45	16.5	16.3	15.2	13.7	130 (35 <i>P. yonahlossee</i> , 60 <i>P. jordani</i> , 25 <i>P. glutinosus</i> , 10 <i>P. c. cinereus</i>)
6/1/68	20:50	15.5	15.2	15.0	13.0	38+ (20 <i>P. yonahlossee</i> , several <i>P. jordani</i> , 18 <i>P. glutinosus</i>)
5/30/68	21:00	12.0	11.5	11.1	11.0	15+ (5 <i>P. yonahlossee</i> , several <i>P. jordani</i> , 10 <i>P. glutinosus</i>)
Bat Caves, NC						
6/2/68	13:40	20.0	19.9	18.5	17.0	2 (<i>P. longicrus</i>)
7/26/68	11:25	23.7	22.4	20.6	19.0	2 (<i>P. longicrus</i>)

^aThe notation "several" indicates that many *P. jordani* were observed but not collected.

agreement with those of Pope and Pope (1951). They found no difference in habitat between *P. ouachitae* and *P. glutinosus*, but suggested that *P. caddoensis* had a greater tolerance of dry conditions. The main habitat difference observed in this study among the plethodonts in the Ouachita Mountains was an apparent difference in the moisture content of the substrate at their capture sites. Casual observation indicated that *P. c. serratus* occupied the driest substrate, followed by *P. caddoensis*, *P. ouachitae*, and *P. glutinosus*. In the Ozarks *P. glutinosus* occupied sites along the margin of the forest floor and cave-ledge habitats, whereas *E. lucifuga* was associated with caves and ledges.

In general my observations on the ecology of the salamanders found along Old Yonahlossee Road are in agreement with those of Pope (1950) and Gordon, MacMahon, and Wake (1962). *Plethodon yonahlossee* was common in most areas along the road and was usually collected in drier microhabitats than *P. glutinosus*. *Plethodon jordani* was the most abundant salamander at this location and appeared to tolerate the greatest extremes of temperature and humidity. It was active sooner after dark and on colder nights than *P. yonahlossee*. Selection of dry microhabitats by *P. c. cinereus* appeared to reduce

competition between it and the large plethodonts. Bogert (1952) reported that *P. c. cinereus* was more abundant than *P. glutinosus* in the drier of two localities in southwestern Virginia.

Desmognathus monticola was more closely associated with water than *D. ochrophaeus*. The latter species wandered about on the forest floor far away from the nearest stream. This observation agrees with that of Hairston (1949) who stated that 54% of the *D. monticola* he collected were from the non-inundated portion of a stream bed, but 76% of the *D. ochrophaeus* were terrestrial.

Observations on the ecology and habitat of *P. longicrus* were essentially the same as those of Adler and Dennis (1962). Individuals were taken from crevices in large rockfaces, but were not present in crevices with a soil substrate. Substrate temperatures (22.1°C and 20.7°C) recorded at the capture sites of two large adults suggest that this salamander encounters slightly higher microhabitat temperatures than the *P. yonahlossee* on Grandfather Mountain.

GENERAL DISCUSSION

Distributional patterns

Differences in the distribution patterns of species within the *yonahlossee* and *glutinosus* groups are

not entirely due to differences in their thermal ecology and water relations. In general the responses of these species were similar in all laboratory tests. *Plethodon glutinosus* did not demonstrate any specific adaptation in its thermal ecology or water relations that would account for its widespread distribution in the eastern United States. Results for the Arkansas populations of *P. glutinosus* were similar to those for the North Carolina populations. Apparently the widespread success of *P. glutinosus* is due more to its ability to survive in generally unfavorable areas through the selection of suitable microhabitats where conditions do not exceed its tolerance limits than to any special physiological adaptation to hot or dry environmental conditions.

Altitudinal distribution of *P. glutinosus* is probably limited in part by competition with other members of the *yonahlossee* and *glutinosus* groups which are better adapted for life at higher elevations. Field studies have shown that the restriction of *P. glutinosus* to lower elevations in the Blue Ridge Mountains is apparently due to competition with *P. jordani* (Hairston 1949, 1951). Highton and Henry (1970) report extensive hybridization among *P. jordani* and *P. glutinosus* in the southern Appalachians and agree with Hairston's conclusion that competition is an important factor in determining the altitudinal distribution of these species. The labile thermal preferendum and decreased sensitivity to atmospheric moisture of *P. jordani* may be adaptations which allow it to survive at both low and high elevations. Its vertical distribution extends from 800 ft on the southeastern escarpment of the Blue Ridge Mountains (Bruce 1967) to 5,900 ft on Grandfather Mountain (Pope 1950). The altitudinal range of *P. yonahlossee* extends from 2,500 ft to 5,700 ft in the Blue Ridge Mountains (Pope 1950); its low thermal resistance may represent an adaptation to the cold temperatures prevalent at high altitudes. These two species appear to be better adjusted physiologically for life at high elevations than *P. glutinosus*.

An additional factor in the restriction of *P. glutinosus* to lower elevations is its association with logs, rocks, and other superficial cover. *Plethodon yonahlossee* and some *P. jordani* retreat to deep burrows during the day (Gordon et al. 1962 and personal observation), and *P. ouachitae* and *P. caddoensis* spend much time beneath the surface of the rocky slopes of the Ouachita Mountains. The inability of *P. glutinosus* to make extensive use of the subterranean portion of a particular habitat may put it at a competitive disadvantage and help restrict its numbers in those areas where the other species are abundant. Likewise the adaptations of the other species to high altitudes may represent specializations that prevent them from invading lowland areas inhabited by *P. glutinosus*. *Plethodon longicrus* occurs at a low

elevation, but is isolated from *P. glutinosus* by its restriction to wet crevices in rockfaces near Bat Caves, North Carolina. Its low heat resistance may restrict it to this habitat where it can escape extreme changes in the ambient temperature by retreating deep into the granite cliffs.

Plethodon ouachitae and *P. caddoensis* have survived in the Ouachita Mountains because of the unique physiographic features of this region and the burrowing behavior of these species. The Ouachita is the only major mountain range in the United States that lies in an east-west direction. Because of their position these mountains receive abundant precipitation via moist air from the south and southwest. North slopes of these mountains are cooler and wetter and support more mesic vegetation than the south slopes (Palmer 1924). By inhabiting these north slopes *P. ouachitae* and *P. caddoensis* encounter microclimatic conditions more like those of the southern Appalachians than of the surrounding lowlands of Arkansas and Oklahoma. By burrowing deep beneath the surface of the talus-covered slopes they escape extreme heat and dryness in the summer months.

Habitat selection and niche segregation

Temperature and water have an effect on the habitat selection and niche segregation of the salamanders tested in this study. Differences in habitat selection of salamanders in the Ouachita Mountains were due primarily to differences in their moisture preferences and water relations. *Plethodon c. serratus* preferred dry microhabitats as was demonstrated in the field data, preferred relative humidity tests, and rehydration studies. *Desmognathus fuscus* was isolated from the plethodonts by its aquatic existence and reduced rate of rehydration in water. *Plethodon caddoensis* and *P. ouachitae* are allopatric species (Blair and Lindsay 1965). *Plethodon caddoensis* occurs at lower elevations in the Novaculite Uplift, a subdivision of the Ouachita Mountain range southeast of Rich Mountain. It has no special adaptations to heat or moisture that permit it to occupy hotter and dryer sites than *P. ouachitae*. In fact, the small size of *P. caddoensis* results in a somewhat faster rate of dehydration than that seen in *P. ouachitae*.

Eurycea multiplicata is the most aquatic of the plethodontid salamanders in the Ozarks, occupying a niche similar to that of *D. fuscus* in the Ouachita Mountains. Its restriction to an aquatic habitat is not due to water-balance problems in the adult stage. It dehydrated at the same rate as *E. lucifuga* and rehydrated at a rate equal to that of *E. longicauda* and *P. glutinosus*. The occurrence of *E. multiplicata* in cool springs and spring-fed streams may be correlated with its low heat resistance, since its CTM is below that of most of the other Ozark plethodontid

species. The aquatic larval stage of *E. multiplicata* may also contribute to the association of adults with water or very wet substrates.

Yellow-and-orange-striped *P. dorsalis* were found primarily in upland oak and pine forests in the Ozarks. Their preference for dry microhabitats is reflected by their low preferred RH and low success in discriminating between levels of atmospheric humidity in the RH gradient. The leadback phase of *P. dorsalis* was found on moist substrates associated with caves. Results of the laboratory tests for these individuals were similar to those for the yellow-and-orange-striped animals.

Niche overlap between *E. lucifuga* and *P. glutinosus* is minor because they occupy distinctly different microhabitats. *Eurycea lucifuga* occupies caves and wet ledges, whereas *P. glutinosus* occupies sites on the forest floor. *Eurycea longicauda* is rare in the presence of the other two species, since it is found only at the junction of the forest floor and cave-ledge habitats. Its numbers may be reduced as a result of competition with *P. glutinosus* and *E. lucifuga*. Differences in the thermal relations of these species are not responsible for the observed differences in their niches. The higher preferred temperatures and greater heat resistance of the *Eurycea* are not reflected in their habitat selection. Differences in the water relations of these species are related to their niche segregation. The slower dehydration rate of *P. glutinosus* allows this species to occupy forest-floor microhabitats that are drier than those associated with the cave-ledge habitat. The greater success of *E. lucifuga* and *E. longicauda* in the humidity gradient may indicate an adaptation to conserve water. Their ability to discriminate between different levels of atmospheric moisture would lessen their exposure to low humidities and reduce their water loss by restricting their activity to periods when the VPD was low.

Differences in the habitat selection of salamanders along Old Yonahlossee Road are related to differences in their temperature tolerances and water relations. *Desmognathus ochrophaeus* and *D. monticola* have a lower heat resistance, slower rate of rehydration, and less success in the RH gradient than the plethodonts. *Desmognathus monticola* is closely associated with water, whereas *D. ochrophaeus* is semi-terrestrial, inhabiting areas near small streams. Apparently little, if any, microhabitat overlap between *Desmognathus* and *Plethodon* is found at this location.

The area around Old Yonahlossee Road is one of the few sites where *P. yonahlossee*, *P. jordani*, and *P. glutinosus* are found together in large numbers. Usually one of these species is absent since *P. glutinosus* seems to be confined to lower elevations and *P. jordani* occupies higher elevations in the southern

Appalachians. This pattern is evident on White Top Mountain in southwestern Virginia (Pope 1950) and in the Black Mountains northeast of Asheville, N. C. (Hairston 1949). The unique situation along Old Yonahlossee Road indicates that favorable environmental conditions exist for all three species and suggests that there are other well-defined differences in their niche requirements. In general the responses of these three species to the laboratory tests were similar. Differences were not of sufficient magnitude to account for the segregation of these plethodonts along Old Yonahlossee Road.

Field studies indicated that *P. glutinosus* occupied wetter areas and was more active on wet nights than *P. yonahlossee*. The nocturnal activity peaks reported for these species by Gordon et al. (1962) were not confirmed in this study. The relative abundance of plethodonts on different nights in the spring and summer of 1968 and 1969 was due more to differences in temperature, moisture, and wind speed than to temporal differences in the activity periods of these species. Apparently *P. yonahlossee*, *P. glutinosus*, and *P. jordani* were isolated by differences in their microhabitat preferences and in their responses to temperature and moisture levels in the study area.

The performance of *P. c. cinereus* in the laboratory tests was similar to that of the other plethodonts collected along Old Yonahlossee Road. Field observations indicated that this species was isolated from the other plethodonts due to its selection of drier microhabitats under superficial cover.

Bioenergetics, activity, and water loss

To occupy a given habitat successfully a species must be able to obtain sufficient energy for growth, maintenance, and reproduction. A salamander population will be unable to survive in a locality where its food-seeking activity is restricted to the extent that most individuals are in negative energy balance. Favorable temperature and moisture conditions must be present during a sufficient portion of the year so that the salamanders can obtain enough food to satisfy their energy requirements. If high temperatures and dry conditions persist for an extended period, the surface activity of salamanders will be restricted. If they normally obtain most of their food above ground, the point may be reached where energy requirements exceed energy intake.

Female *P. glutinosus* in Maryland and Pennsylvania and female *P. cinereus* in Maryland deposit eggs only once every 2 years (Highton 1962a, Sayler 1966). These animals may not obtain sufficient energy to allow reproduction to occur each year. This may be due to a reduction in activity because of unfavorable environmental conditions and a consequent decrease in energy available for reproduction. *Plethodon glutinosus* in Florida lay eggs once a year (Highton

1956). These salamanders may encounter more favorable climatic conditions and accumulate energy stores faster than the northern populations of this species.

Spight (1967a) stated that salamanders can obtain water from agricultural land in North Carolina throughout most of the year. While this may be true, salamanders will be unable to remain active in areas where their daily water loss resulting from activity exceeds their daily water uptake from the soil. In this situation they will be unable to maintain their water balance unless they restrict their nocturnal activity and remain beneath the surface to replenish the water lost during their last activity period. Ultimately increased dehydration resulting from nocturnal activity at high temperatures and low humidities will force them to extend the time spent in rehydration and curtail that spent in surface activity. When the quantity of water lost in one night's activity reaches a critical level these salamanders must cease their aboveground activity and remain underground until the microclimatic conditions become less severe.

With the results that I obtained it may be possible to determine the point at which the water loss of a salamander during nocturnal activity would exceed the water uptake during the remaining portion of the day. This point will indicate the critical levels of temperature and moisture beyond which the salamander's activity will be restricted. By correlating this information with data on the climate and microclimate of a particular location it would be possible to calculate the number of nights per year that the salamander could be active at that site. If the metabolic requirements of the species were known and information were available on its food intake and assimilation efficiency and on the caloric value of the food, it would be possible to predict whether or not that species could remain in energy balance in that location. If the available data were precise enough it might even be possible to predict the frequency of reproduction for individuals of the species in that location since egg-laying frequency would be related to the rate of accumulation of excess energy by these individuals.

Plethodon caddoensis is the smallest of the eastern large plethodonts. Its activity may be curtailed by the high temperatures and lack of moisture common in the Novaculite Uplift during the summer months. At 25°C with a RH of 71% *P. caddoensis* would lose 25.0 mg cm⁻² in 8 hr of surface activity. At a soil-moisture tension of 0.33 atm (normal tension after gravitational water has drained away) it would regain only 10.4 mg cm⁻² in the next 16 hr and would suffer a water deficit of 14.6 mg cm⁻². If *P. caddoensis* were active for 3 hr at a RH of 65% at 25°C its water loss would be 10.5 mg cm⁻². At a soil-moisture tension of 0.33 atm it could regain

13.6 mg cm⁻² in the next 21 hr and would not suffer from a dehydration deficit during its next activity period. Assuming the same soil-moisture tension and temperature *P. caddoensis* could be active for 4.5 hr at 80% RH, 5.8 hr at 90% RH, and 7.5 hr at 100% RH. At high temperatures and moderate humidities the aboveground activity of this species is greatly curtailed. On June 17, 1968, nocturnal microclimatic temperatures at Camp Albert Pike ranged from 22.0° to 23.5°C and only one salamander was collected. The critical temperature level for this species is reached in early summer, and salamander activity is severely limited until the temperature at this location declines in the fall. The abbreviated activity period available to this species during the summer months may be too short to provide for the assimilation of sufficient energy for continued growth. This seasonal reduction in energy uptake may result in a shortened growing season and could be responsible for the small size attained by adult *P. caddoensis*.

Eurycea lucifuga in the Ozarks inhabits caves and wet ledges. At 25°C with a RH of 90% it would lose 17.7 mg cm⁻² in 6 hr of surface activity. At a soil-moisture tension of 0.33 atm it would regain only 11.7 mg cm⁻² in the next 18 hr and would suffer a water deficit of 6.0 mg cm⁻². In 3 hr of surface activity at 25°C with a RH of 66.6% *E. lucifuga* would lose 13.2 mg cm⁻² water. In the next 21 hr it could take up 13.6 mg cm⁻² of water from the soil at a moisture tension of 0.33 atm and would not undergo a dehydration deficit. Assuming the same soil-moisture tension and temperature *E. lucifuga* could be active for 3.3 hr at 70% RH, 3.6 hr at 80% RH, and 5.5 hr at 100% RH.

Plethodon jordani occupies higher elevations in the southern Appalachians. At 0.33 atm soil-moisture tension with a temperature of 25°C it takes up water at a rate of 0.55 mg cm⁻² hr⁻¹. Thus it could be active aboveground for 6 hr at 100% RH, 4.8 hr at 90% RH, 3.8 hr at 80% RH, and 3.3 hr at 70% RH.

At 25°C these two species could be active for similar lengths of time at moderate humidities. Their distribution may be limited in part by their shortened activity periods at higher temperatures. In warmer microhabitats these species may be unable to obtain sufficient energy for maintenance and growth during the summer months. Thus these salamanders would be in negative energy balance and could not survive in such habitats. Other species may also be limited in distribution by their inability to remain active for sufficient periods of time to obtain adequate energy supplies for growth and reproduction.

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