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Experimental Studies on Habitat Preference and Thermoregulation of Bufo americanus, B. hemiophrys and B. cognatus

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ABSTRACT—This paper reports on responses to habitat and thermoregulation exhibited by three species of toads, *Bufo americanus*, *B. hemiophrys* and *B. cognatus*, under artificial conditions. The response of the toads to sparse, medium, and dense vegetative cover was investigated by establishing artificial cover types in a sheet-metal enclosure. Each of the three species spent more time in the dense cover than in sparse or medium cover. A second experiment to determine if the toads would be attracted by odor to samples of mud, water, and vegetation from their respective ranges suggested that the toads moved randomly to the habitat samples. The role of temperature-regulating ability in habitat selection was evaluated by measuring deep body temperatures of the toads and surface temperature of the substrate under varying environmental conditions. The relations between body and surface temperature were significantly different for the three species as shown by analysis of covariance. *B. americanus* exhibited the most and *B. cognatus* the least thermoregulation. The possible roles of these factors in causing the present distribution patterns of the three species are considered.

The distributional limits of each species are determined by its tolerances to certain physical and biological factors. In addition, most species are further restricted to certain microenvironments within the limits fixed by these tolerances. Environmental factors that correlate with specific distribution patterns have frequently been reported without evidence substantiating a cause and effect relation. The ultimate factor or factors determining the distribution patterns of most organisms are unknown. The unique nature of the distribution of three toads, *Bufo americanus*, *B. hemiophrys*, and *B. cognatus*, in northwestern Minnesota, has led us to investigate possible ultimate causative factors. This paper reports on responses to habitat and thermoregulation exhibited by these three species under artificial conditions.

The ranges of the toads in northwestern Minnesota (Fig. 1) appear to be related to the gross physiognomy of the region. Breckenridge (1944) and Tester and Breckenridge (unpublished data) have shown that in Becker and Mahnomen Counties the western limit of *Bufo americanus* coincides with the western edge of the Big Stone Moraine, an irregular north-south belt of hills.

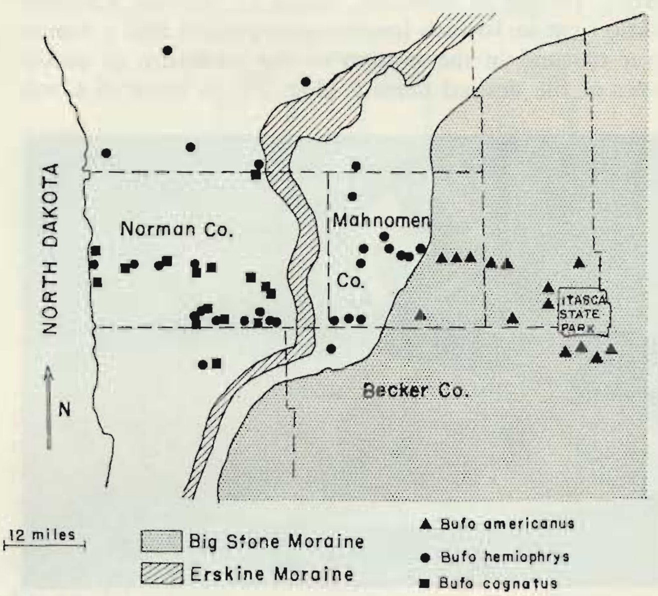

FIG. 1. Collection sites of *Bufo americanus*, *Bufo hemiophrys*, and *Bufo cognatus* in northwestern Minnesota (Moraine locations from: Leverett, F. and F. W. Sanderson. 1932. Map of the northern part of Minnesota showing surficial deposits.)

The eastern limit of *B. hemiophrys* follows this same margin. No overlap of the two ranges has been discovered. Approximately 25 miles to the west of this moraine *B. cognatus* reaches its eastern limits along the old beach lines of Glacial Lake Agassiz. From this area westward *B. hemiophrys* is sympatric with *B. cognatus*.

All three species are capable of extensive movement across uplands (Breckenridge and Tester, 1961 and unpublished data). In spite of this capability and the fact that the habitat appears to be similar both east and west of the Lake Agassiz beach lines, *Bufo cognatus* has not extended its range eastward into the narrow zone now occupied exclusively by *Bufo hemiophrys*.

The results of preliminary studies of the effects of water chemistry and temperature on survival of tadpoles of

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B. americanus and *B. hemiophrys* and the effects of known differences in air temperature, precipitation, and soil chemistry on the distribution of adults have been reported by Tester and Breckenridge (1963). Their data suggested that the factors under investigation were not those limiting the distribution of the three species. These negative results led to the present study on habitat preference.

Methods

The experimental studies were conducted at the Lake Itasca Forestry and Biological Station during June and July, 1963. The station is located in the hardwood-conifer forest ecotone in Clearwater and Hubbard Counties, Minnesota, and is approximately 35 miles east of the transition between the hardwood forest and the prairie.

The response of the toads to sparse, medium, and dense vegetative cover was investigated by establishing artificial cover types in a sheet metal enclosure approximately 10 feet in diameter. Stems of bullrush (*Scirpus acutus*) cut in 16-inch lengths were pushed into a 4-inch layer of sand in the bottom of the enclosure to create cover of the desired density (Fig. 2). A layer of 6 mil

FIG. 2. Test enclosure for investigating response of toads to sparse, medium and dense vegetative cover.

polyethylene sheeting was placed on the ground before the sand was poured into the enclosure to prevent native vegetation from growing through the sand and contributing to the experimental cover.

The enclosure was divided into three equal sectors of varying cover density. The segment oriented north of center had stems placed 0.5 to 1.0 inch apart simulating dense vegetative cover. Stems were placed 2 to 3 inches apart in the southwest segment and 4 to 5 inches apart in the southeast, simulating medium and sparse cover, respectively. When approximately half the experiment had been completed the enclosure was cleaned and new bullrushes were placed so that the compass orientation of the segments was reversed.

A 12-inch diameter area in the center of the enclosure was kept open and used as the release point for all tests. One or two toads of each species were released simultaneously for each test. The positions of these toads at the end of each minute during the 30-minute test period were recorded and notes were made on behavioral characteristics of the three species.

In a second experiment, tests were made to determine if the toads would be attracted by odor to samples of mud, water, and vegetation from their respective ranges as suggested by Martof (1962). A test apparatus (Fig. 3)

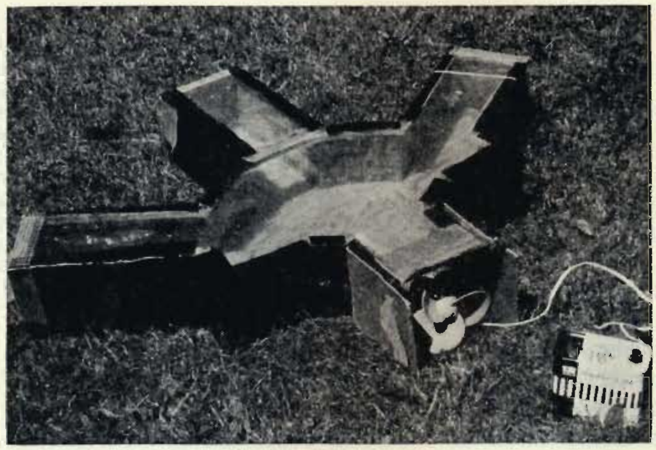

FIG. 3. Test chamber for investigating olfactory response of toads to samples of mud, water and vegetation.

with three response compartments was constructed so that odors from the three samples would pass through the area where the test animal was introduced. The animal would then be free to move to the compartment containing the mud-water-vegetation sample of its choice. An exhaust fan was placed at the end of the chamber opposite the test compartments to provide for a continuous air flow of 15 feet per minute during the experiments. Toads were placed in the chamber in front of the exhaust fan and their initial response pattern was recorded. An individual experiment was concluded when the animal moved into one of the three test compartments. Samples were reoriented randomly after each series of six tests and no toads were tested more than twice in one day.

In an effort to evaluate the role of temperature-regulating ability in habitat selection, the deep body temperature of the toads and surface temperature of the substrate were measured under varying environmental conditions. All temperature readings were taken with a quick-reading Schultheis thermometer. Deep body temperatures were obtained by inserting the bulb of the thermometer into the cloacal opening while the toad was sitting in a normal position. The substrate surface temperature was taken immediately after the deep body reading by laying the thermometer horizontally on the substrate. We believed that this temperature rather than ambient air temperature was more pertinent to the objective since the toads were nearly always in direct contact with soil surface and were receiving radiant energy directly. Scott and Carpenter (1955) found that cloacal temperature of *Bufo woodhousei* showed a closer relation to ground surface temperature than to air temperature, taken at three feet above the surface, and Rosenthal (1957) considered the air-surface interface temperature rather than the free air temperature indicative of the environment of the salamander, *Aneides lugubris*.

Results

Relation of vegetation density to choice of area

Two measurements were taken to determine if the three species demonstrated different preferences for sparse, medium, or dense vegetative cover. These were the total time in minutes that each toad spent in each density type during a 30-minute test period (Table 1), and the type each specimen was in at the end of this period (Table 2).

Table 1. Chi-square test of independence between species and time in minutes spent in each cover density.

	Cover density						
	Dense		Medium		Sparse		
Species	Observed	Expected	Observed	Expected	Observed	Expected	Total
Bufo cognatus	671	720.6	294	322.4	390	311.9	1831
Bufo hemiophrys	873	911.6	476	407.9	365	394.6	1714
Bufo americanus	1063	973.8	396	435.7	373	421.5	1355
Total	2606		1166		1128		4900

Chi-square = 54.41** Significant at .01 probability level.

Table 2. Chi-square test of independence between species and location in cover types at end of 30-minute test period.

	Cover density						
	Dense		Medium		Sparse		
Species	Observed	Expected	Observed	Expected	Observed	Expected	Total
Bufo cognatus	24	23.2	8	10.3	9	7.5	41
Bufo hemiophrys	38	40.1	18	17.8	15	13.0	71
Bufo americanus	46	44.7	22	19.9	11	14.5	79
Total	108		48		35		191

Chi-square = 2.37. Not significant at .05 probability level.

Chi-square tests were used to evaluate the relation of species to cover density. The first hypothesis tested was whether species preference was independent of cover density (Table 1). The Chi-square of 54.41 is highly significant (.01 probability level) indicating that these three species demonstrated different preferences for the three cover types.

The second hypothesis tested was whether location at the end of 30 minutes was independent of species influence (Table 2). This hypothesis was not rejected (Chi-square = 2.37). Therefore, it is probable that at this point in time, cover type chosen had no relation to species.

The results of these two measures of the relation of species to vegetation type seem contradictory and we can only speculate as to what the actual situation may be. During the first minutes of occupancy of the pen, it is possible that the toads may have been orienting themselves relative to environmental factors other than cover density; i.e., shade, sun, and perhaps air currents. It may be that such factors influenced cover-type selection since the tests were carried on for several days and it was impossible to have each individual exposed to the same physical environment. Since the species were found to be independent of location in a particular cover density at the end of the test period, it seems possible that the shift observed was not a species response, but one caused by a cumulative effect of one or more environmental factors.

Olfactory response

An attempt was made to determine experimentally whether these three species of toads might show olfactory response to mud-water-vegetation habitat samples typical of their particular ranges. Table 3 shows the number of moves by the toads to the three habitat samples used in this experiment. Chi-square analysis was used to test the hypothesis of whether species type was independent of habitat sample chosen. Chi-square was 8.39 and the hypothesis was not rejected at the .05 probability level. On the basis of these data we have no reason to believe the toads moved other than randomly to the habitat samples.

Table 3. Chi-square test of independence between species and mud-water-vegetation types chosen by olfactory response.

	Source of mud-water-vegetation sample						
	Bufo cognatus range		*Bufo americanus* range		*Bufo hemiophrys* range		
Species	Observed	Expected	Observed	Expected	Observed	Expected	Total
Bufo cognatus	7	11	16	13	13	12	36
Bufo hemiophrys	11	11	9	13	16	12	36
Bufo americanus	15	11	14	13	7	12	36
Total	33		39		36		108

Chi-square = 8.39. Not significant at .05 probability level.

Thermoregulation

Paired measurements of deep body temperature and substrate surface temperature were taken of 30 individuals of each species (Fig. 4) when the toads were in holding cages or in the cover density enclosures. An attempt was made to obtain only mature individuals for this experiment. The size at which each of the three species becomes mature is not definite; however, since 45 mm appears to be the size at which *Bufo hemiophrys* matures (Breckenridge and Tester, 1961) this size was arbitrarily selected. A few specimens of *Bufo cognatus* were used which were slightly smaller because of the scarcity of this species during the period of investigation.

The relation between deep body temperature and surface temperature was assumed linear and regression equations were computed (Fig. 4). Results of this analysis suggested that the slopes of the regression lines were different. To determine whether an actual difference between slope for each species did exist, the hypothesis of

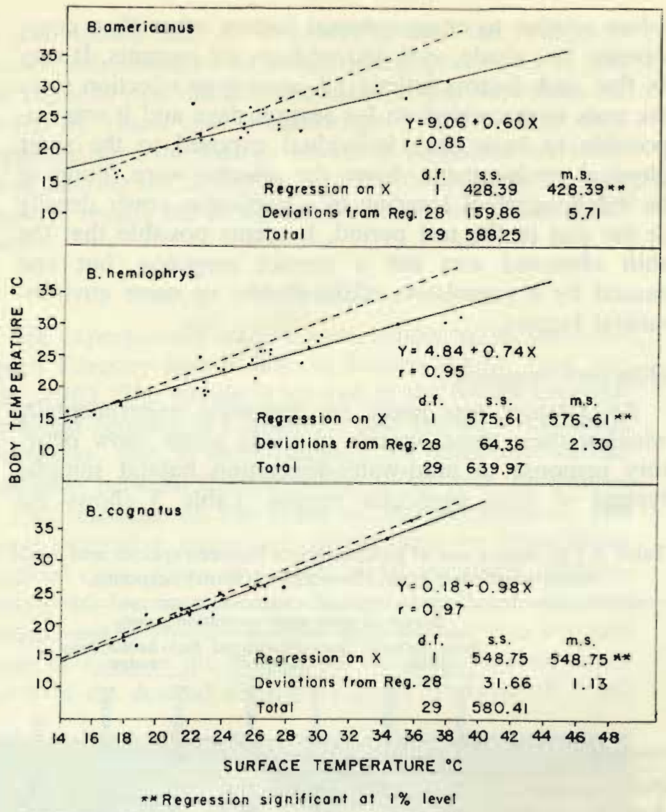

FIG. 4. Relationship between surface and body temperatures of *Bufo americanus*, *Bufo hemiophrys* and *Bufo cognatus*. Broken line represents the equation, × = y. (See text for explanation).

homogeneous slope was tested by covariance analysis. However, one of the basic assumptions of covariance analysis is that the residual variances are homogeneous. It was observed that the residual variance, represented in this case by the mean square for deviations from regression, for each species appeared quite different (Fig. 4). Bartlett's (1937) test for homogeniety of variance was used and the differences in variance were found to be significant at the .01 probability level. A log transformation was made so that the data would satisfy the requirements for the analysis of covariance.

The analysis of covariance (Table 4) indicates a difference in slope (.05 probability level), which suggests that a different relation exists between body temperature and surface temperature for these three species. A visual comparison of the body-surface temperature relations of the three species can be made from the plots of the original data and the lines of the regression equations in Figure 4. All three regressions are significant at the .01 probability level.

The regression for *Bufo cognatus,* as shown by the solid line in the lower part of Figure 4, has a slope very close to one, whereas the other species demonstrate slopes somewhat less than one. In addition, Figure 4 shows that the scattering about the regression line is greatest for *Bufo americanus* and least for *Bufo cognatus*. This phenomenon is made more apparent by comparing the deviations from regression mean squares, which are expressive of the total amount of variation from each regression equation.

Discussion

The results of the cover density study did not show that *Bufo americanus* "preferred" the dense cover simulating woods habitat and that *Bufo cognatus* and *Bufo hemiophrys* "preferred' the sparse cover simulating grassland habitat. Each of the three species spent more time in the dense cover than in sparse or medium cover (Table 1). This may have been a real response to the simulated habitat, or it may have been an escape response related to the stresses of captivity, or to the sheet metal enclosure.

Individual toads may have moved about within the enclosure searching for "optimum conditions" or trying to escape, but returning frequently to the dense cover type. If this was true, their position at the end of the test period or at any specific time would have a high possibility of being random. This might account for the low Chi-square for the data on location at the end of the 30-minute test (Table 2).

The artificial cover densities used in these experiments were designed to evaluate the role of visual or tactile stimuli provided by the spacing of the stems of bulrush. This design simulated only upland characteristics and was highly artificial in that no litter or mulch cover was provided over the sand. It may be that different re-

Table 4. Covariance analysis testing differences in slope for the relationship between body temperature and surface temperature for three species of *Bufo*.

Source	Degrees of Freedom	Sum of Squares for X	Sum of Cross Products	Sum of Squares for Y	Corrected Degrees of Freedom	Reduced Sum of Squares	Mean Square
Bufo americanus	29	1.57893	1.17487	1.09097	28	0.21676	
Bufo hemiophrys	29	1.77831	1.43401	1.27212	28	0.11575	
Bufo cognatus	29	0.95534	0.93237	0.97304	28	0.06308	
Within					84	0.39559	0.00471
Common Regression	87	4.31258	3.54126	3.33614	86	0.42826	
Regression Coefficients					2	0.03266	0.01633

Test for difference in slope: $F = 3.468^*$ with 2 and 84 degrees of freedom; significant at .05 probability level.

sponses would occur under more natural conditions in which both aquatic and terrestrial habitats would be available.

The movement of the toads to the mud-water-vegetation samples indicates that olfactory response was independent of a species-habitat relation. Martof (1962) found in laboratory experiments that *Pseudacris triseriate* demonstrated a preference for muck and algae from its breeding habitat. It should be noted that our tests were not conducted during the breeding season but that the habitat samples were obtained from known breeding areas.

Even if an olfactory preference was exhibited during the breeding season, one might expect the toads to move to other areas, i.e., expand their range, at other times of the year. We have not observed movements of this type nor have we found species "outside" their ranges during the nonbreeding season. These findings suggest that olfactory response is not a factor influencing the distribution of these species.

The ability of amphibians to regulate their body temperatures within certain limits and the possibility that each species exhibits a preference for a certain temperature (Brattstrom, 1963; Fitch, 1956; Kirk and Hogben, 1946; Scott and Carpenter, 1955) provide potential mechanisms controling geographic distribution.

Our data indicate that under conditions of high ambient temperatures, up to 46° C, *Bufo americanus* maintains the lowest body temperature, *Bufo cognatus* has a body temperature near ambient, and *Bufo hemiophrys* is intermediate (Fig. 4). Under our experimental conditions, where all species were measured in the same artificial habitat, it appears that control is due to evaporative cooling. This explanation is supported because the data for *Bufo americanus*, the species that exhibited the most regulation, show the greatest variability, whereas, the least scattering about the regression line occurs for *Bufo cognatus*, the species that showed the least thermoregulation.

It seems likely that temperature preference, if it exists, would be an important factor in determining distributional limits of these toads. It is also probable that thermoregulation would be used to maintain preferred body temperatures under certain conditions. Although we do not have data on preferred temperatures, it is worthwhile to speculate on the role of temperature in the geographic distribution pattern in the region studied.

If *Bufo americanus* preferred a "cool" temperature, such as that found in the shaded forest, it would be required to evaporate a large amount of water or to employ behavioral mechanisms or both to maintain this "cool" body temperature, if it were to move westward into the prairie. The amount of evaporative cooling required under prairie conditions might exceed the physiologic capabilities of the species and therefore could lead to death. This might explain why *Bufo americanus* has not extended its range westward onto the prairie. However, Schmid (1965) found that *Bufo americanus* had approximately the same resistance to artificial desiccation as *Bufo hemiophrys*.

The apparent lack of temperature regulation in *Bufo cognatus* suggests that either this species does not find the higher temperatures "uncomfortable" or that it does not possess as much thermoregulatory capability as the other two species. Neither alternative offers further explanation regarding the lack of range extension to the east for this species.

Incidental ethological observations were made while the toads were in the cover density enclosure. These findings, although not quantitative, provide substantiating evidence for the above conclusions. Our observations revealed that in the range of about 25° to 30° C all three species appeared quite "content" in the pen and would often sit for long periods feeding on ants and other insects. Fitch (1956) stated that the preferred temperature range of *Bufo americanus* is from 26° to 31° C and Scott and Carpenter (1955) reported a mean body temperature of 26.4° C for 141 readings from *Bufo woodhousei*, a closely related species.

At temperatures above approximately 38° C, *Bufo cognatus* usually burrowed and *Bufo americanus* appeared "nervous" and attempted to crawl up the enclosure wall. *Bufo hemiophrys* usually moved to shade and remained quiet.

The behavior of the species changed markedly when temperatures dropped below 30° C. *Bufo cognatus* became very active and made many attempts to jump over the sheet metal whereas *Bufo americanus* appeared "content." At times *Bufo hemiophrys* seemed "content" and at other times it attempted to crawl out of the enclosure.

These observations on behavior under conditions that might cause stress suggest that *Bufo cognatus* can exist under higher environmental temperatures than *Bufo americanus*. The "nervous" reaction of *Bufo americanus* under high temperature stress, and its capacity for thermoregulation, may be related and may combine to limit its distribution to "cool" areas. It appears that this species may have a real need for a cooler environment since it does not exhibit burrowing behavior that would allow it, at least temporarily, to escape the heat stress.

The data presented here do not offer any explanation for the absence of *Bufo cognatus* and *Bufo hemiophrys* in the forest biomes in northwestern Minnesota.

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Dr. — Yes or No? — *(Continued from page 26)*

distinction. As an extension of this idea some even feel that Ph.D.'s should not address or refer to one another by title. While Benjamin Franklin was addressed as "Dr. Franklin," and while this certainly is proper — if maybe somewhat formal — usage, I think titles denoting distinction are gradually disappearing. Being addressed as Mr. puts one into rather good company: the *Congressional Record* refers to senators as Mr., and Gen. Eisenhower would have never become Mr. Eisenhower if it weren't for his promotion at the polls.

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The title "Dr." is much overused in our society and often does not in fact represent the level and type of academic or professional training that many people associate with it. As Shaw wrote in 1903 in *Man and Superman*, "Titles distinguish the mediocre, embarrass the superior, and are disgraced by the inferior." Degrees are clearly not becoming obsolete. Nevertheless, it is time for a reexamination of the effects that titles — that is, the symbols, as distinguished from the referents — are having on social behavior.

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