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Upper Thermal Tolerances of Anuran Embryos in Relation to Stage of Development and Breeding Habits

RICHARD G. ZWEIFEL[1]

ABSTRACT

Embryos of several species of anurans were exposed from two to 10 hours and at various stages of development to higher temperatures than the maximum that early embryos of the same species can tolerate under continuous exposure. The purposes were to determine (1) the extent of increase in temperature tolerance through ontogeny (2) the period(s) of development when the increase takes place, and (3) the influence of duration of exposure to high temperature on survival. Species studied were a forest-dwelling form adapted to breeding in cold ponds in early spring (*Rana sylvatica*), a stream and pond dweller of southwestern mountains (*Rana* sp., *pipiens* complex), and three species of desert and grassland habitats (*Bufo cognatus*, *Scaphiopus bombifrons*, and *S. couchii*). Embryos studied (and presumably of all anurans) increase their temperature tolerance as they grow. A marked increase takes place early in development, during the first several cleavages. The species of *Rana*, adapted to cooler conditions, do not attain maximum tolerance until gastrulation is well under way or virtually is complete. At the other extreme, *S. couchii* achieves more than 90 percent of its total tolerance before gastrulation has commenced. The highest tolerance among species studied is that of *Bufo cognatus*, whose embryos survive six hours at 40.5° C. when exposed as mid-gastrulae (lethal level was not determined). Species of *Scaphiopus* do almost as well, whereas limits of the two species of *Rana* are several degrees lower. Among most species, the maximum temperature tolerated and the duration of exposure are inversely related, but in *Bufo cognatus* duration of exposure had no obvious effect on maximum tolerance. In its extremely rapid embryonic development, early attainment of maximum tolerance, and high level of tolerance, *Scaphiopus couchii* is the species best adapted to breeding in warm waters. Although adapted to breeding in cold waters, *Rana sylvatica* nevertheless undergoes sufficient ontogenetic expansion in tolerance quickly enough to protect developing embryos against all but the most unseasonally warm temperatures occurring in its breeding ponds.

INTRODUCTION

The importance of temperature in the ecology of embryos of frogs and toads, long evident to naturalists, was placed in a more precise context by Moore and later workers (Moore, 1939, and subsequent papers; see Zweifel, 1968, for citations). Experimenters typically investigated

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rates of development at different temperatures and determined the upper and lower temperatures that limit development. In studies of tolerances, frog embryos at the earliest stages of development (at or before first cleavage) were exposed to various constant temperatures and the range within which normal development occurred was determined. Species were found to differ in upper and lower temperature tolerances and in ranges of tolerance. Differences correlated well for the most part with differences in breeding habitat, geographic distribution, and breeding season. Some widely distributed species showed no evident geographic variation in limiting temperatures: *Rana catesbeiana* and *R. clamitans* (Moore, 1942); *Rana sylvatica* (Herreid and Kinney, 1967; Moore, 1939); *Scaphiopus couchii* (Zweifel, 1968). At least three species do exhibit such variation: *Bufo americanus* and *B. woodhousii* (Volpe, 1953); *Hyla regilla* (Brown, 1975). Apparent geographic variation in *Scaphiopus hammondii* (Brown, 1967b) reflected the inclusion of two species within that taxon (Brown, 1976), and geographic variation in *Rana pipiens* (Moore, 1949) is partly but not wholly explained in that several sibling species were included within *pipiens* (Pace, 1974).

Work of the sort described above, when tolerances are determined under standardized conditions, is valuable in that it provides data on adaptations to temperature that are readily comparable from species to species. In effect, a "base-level" measure of temperature adaptation is obtained. Yet, embryos developing under natural conditions are exposed to fluctuating temperatures, and it is desirable to know how tolerance levels over short periods compare with those at constant temperatures. Also, it has long been known (though evidently not by some who experimented at constant temperatures) that the upper thermal tolerances of anuran embryos increase markedly during ontogeny. Thus, constant temperature experiments begun after embryos have developed for several hours yield different results from those begun at the "standard" time —at or just before first cleavage.

The experiments described here were designed to show the upper thermal tolerances of embryos of a variety of species when exposed to high temperatures for varying periods and at various stages of development. This would, then, reveal (if such exist) interspecific differences in the rate through ontogeny at which increases in tolerance take place as well as differences in the relative and absolute amounts of increase in tolerance. The ecological significance of increased embryonic temperature tolerances could then be assessed.

Although investigators have reported small-scale attempts to determine the influence of briefly elevated temperatures on survival of anuran embryos (e.g., Schectman and Olson, 1941; Herreid and Kinney, 1967), there is only one published report of a thorough, well-planned and well-executed series of experiments: Brown's (1967a) study of the western spadefoot, *Scaphiopus multiplicatus.* My experiments follow essentially the same method as his (see below).

I worked at two facilities of the American Museum of Natural History: the Kalbfleisch Field Research Station near Huntington, Suffolk County, Long Island, New York (short periods in March and April, 1971-1975); the Southwestern Research Station, Portal, Cochise County, Arizona (summers of 1972-1974). I thank the directors of these field stations, respectively Dr. Wesley Lanyon and Mr. Vincent Roth, for the many courtesies extended during my residence. I am grateful to Dr. John A. Moore for his helpful criticism of the manuscript.

MATERIALS AND METHODS

The principal materials of these experiments consisted of embryos of five species of anuran amphibians: *Scaphiopus bombifrons* and *S. couchii* (family Pelobatidae), *Bufo cognatus* (family Bufonidae), *Rana* sp. [*pipiens* species group] and *Rana sylvatica* (family Ranidae). Two other species, *Scaphiopus multiplicatus* and *Gastrophryne olivacea* (family Microhylidae), received less attention.

Embryos were obtained in three ways: by collection of egg masses in the field; by bringing animals found in amplexus into the laboratory where oviposition and fertilization took place; and by inducing oviposition through injection of homospecific pituitary glands, followed by fertilization in a sperm suspension made by macerating testes in stream water. Details of the sources of embryos are in the individual species accounts.

The three intentional variables in the experiments were temperature, duration of exposure to elevated temperature, and stage of embryonic development at which the experiment commenced. Embryos were held at variable room temperatures (or, less often, at a constant temperature well within the normal range tolerated) until they reached the desired starting stage and were then transferred to preheated water in plastic dishes placed in constant-temperature water baths. Depending on their size, the dishes held 400-700 ml. of water. Where there was a considerable difference between holding temperature and experimental temperature, a short time at an intermediate temperature was allowed. The water baths normally maintained the temperature to within less than 0.5° of the set point.[1]

In a typical experiment, embryos from a single clutch of eggs and at essentially the same stage of development were divided among four to six water baths set for a graded series of temperatures. At two, four, six, and 10 hours after the beginning of the run, one-quarter of the original number of embryos in each bath were removed to room temperature. The developmental stage of the embryos (Gosner, 1960) was recorded at the beginning of the experiment, at intermediate times, and as each group was removed from the elevated temperature. Note was made of any evident abnormalities of development, and the temperature in each dish containing embryos was recorded at frequent intervals. After removal to room temperature, developmental progress was followed until the embryos reached stage 20 (gill circulation), unless they died earlier. Stage 20 was taken as the end point of the experiments. Embryos of the species used hatch then or earlier, so under natural conditions they could seek appropriate temperatures. The number of embryos in each time-temperature group varied according to availability but rarely was fewer than 20 and seldom more than 50.

An embryo at the end point, stage 20, was considered normal if it showed typically vigorous swimming movements when stimulated and had no obvious physical abnormalities.

In the accounts that follow, I refer to the highest constant temperature tolerated in experiments initiated at stage 3 (first cleavage) as the base level.

[1] Temperatures are expressed in degrees Celsius.

Voucher specimens (individuals that provided gametes) are deposited in the American Museum of Natural History (AMNH).

Explanation of tables: The columns record the embryonic stage at which the experiment commenced, and each number in these columns indicates the percentage of normal development in one experiment conducted at the time period, temperature, and developmental stage indicated. An exception, in tables 2 and 4, is where more than three experiments were run at a single combination of time, temperature and stage, the mean percentage of normal development is given, followed in parentheses by the range and number of experiments. A dash (–) means that no experiment was run at the nexus of time, temperature and stage. The temperature column indicates the range of temperatures within which the modal temperatures for the experiments fell. That is, temperatures did not necessarily vary over the range indicated, but were centered somewhere within that range.

SPECIES ACCOUNTS

Rana sylvatica Le Conte

Table 1, Figure 1

The wood frog is a terrestrial inhabitant of forested regions, ranging from Alaska through most of Canada and the northeastern United States and south in the Appalachian Mountains (Martof, 1970). The eggs I used came from ponds on or adjacent to the Kalbfleisch Field Research Station. Only eggs oviposited naturally were used, but there is no question as to identity—no other *Rana* breeds in these ponds at the same time as *R. sylvatica.*

Moore (1939), using embryos from the vicinity of New York City, determined that they could endure a constant temperature of 23.7° but not 24.5°. Herreid and Kinney (1967) found high mortality among embryos from the vicinity of Fairbanks, Alaska, raised at 24.3° and set the upper limit for 50 percent survival at about 24°. Thus, the upper thermal limit for constant temperature at stage 3 appears to be the same throughout all or most of the wide range of this species.

Embryos in stage 3 can endure approximately 25.6° (1.6° above base level) for as long as 10 hours and still develop normally, but even two

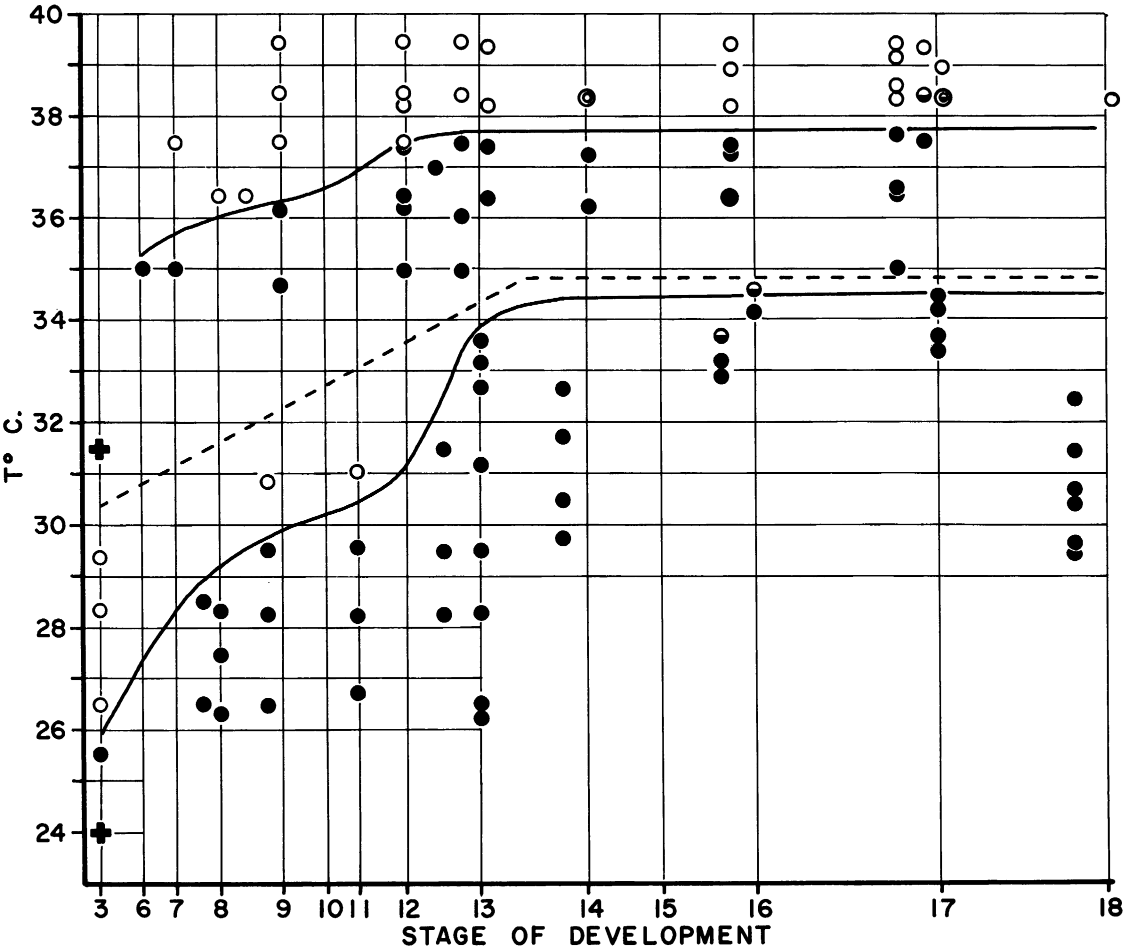

FIG. 1. Ontogenetic change in two-hour temperature tolerance in embryos of *Rana sylvatica* (below broken line) and *Rana* sp. (above). Crosses indicate the base-level tolerances. Each circle represents the results of one experiment: solid circles for normal development, open circles for little or no normal development, half-filled circles for borderline cases. Concentric circles represent two experiments at the same temperature and stage of development. The solid lines are the estimate of the course of increasing temperature tolerance. The Stage of Development axis is adjusted to be roughly linear with respect to time; e.g., it takes longer to pass through stage 16 than through stage 11.

hours exposure to about 26.5° is fatal. By late stage 7 embryos can tolerate 28.5° for at least four hours, and by late stage 8 two-hour tolerance reaches at least 29.5° but not 30.8°. A further slight increase in tolerance (indicated by better survival at four, six, and 10 hours) is evident in embryos commencing gastrulation (stage 11). A marked increase takes place during gastrulation, as embryos in mid-stage 12 tolerated 31.2° (the highest to which they were subjected) for 10 hours, compared with failure in two hours of stage 11 embryos at about 31.3°. By the beginning of stage 13 embryos tolerated 33.7° for four hours, but not longer. The maximum tolerance recorded was 34.6° for two hours in stage 17. Probably this is quite close to the maximum possible, as embryos in early stage 16 had a low percentage of normal development at 34.7°.

The pattern of temperature tolerance manifests itself as a relatively rapid increase in ability to withstand higher temperatures through the early cleavage stages, a much slower rate of increase during late cleavage stages, a marked increase during gastrulation, and little or no in-

TABLE 1

Rana sylvatica: **Percentage of Normal Development After Exposure for Time Shown in Column 2 When in Stages Shown in Columns 3 to 9**

Temp. °C.	Hours Exposed	St. 3	St. 7L-8L	St. 11	St. 12	St. 13	St. 15L-16E	St. 17L
23.9-25.0	2	–	–	–	–	–	–	–
	4	100	–	–	–	–	–	–
	6	–	–	–	–	–	–	–
	10	100	–	–	–	–	–	–
25.5-25.8	2	–	–	–	–	–	–	–
	4	100	–	–	–	–	–	–
	6	–	–	–	–	–	–	–
	10	100	–	–	–	–	–	–
26.2-26.8	2	0	81,100	71	–	71,100	–	–
	4	0	97,98,100	63	–	93	–	–
	6	0	98,98	76	–	100	–	–
	10	0	0[a]	74[a]	–	54[b],100	–	–
27.1-27.7	2	–	100	–	–	–	–	–
	4	36	100	–	–	–	–	–
	6	–	96	–	–	–	–	–
	10	0	–	–	–	–	–	–
28.1-28.7	2	0	87,98	72	100	100	–	–
	4	0	89,93,93	76	97	100	–	–
	6	0	0,98	57	98	–	–	–
	10	0	0[a]	82[a]	100	83[b]	–	–
29.2-29.8	2	0	98	62	96	52,91	–	95,100
	4	0,0	0	55	100	96	–	94,100
	6	0	0	30	100	100	–	94,100
	10	0,0	0[a]	54[a]	100	100	–	94
30.5-30.7	2	–	–	–	–	100	–	77,95
	4	–	–	–	–	86	–	97,100
	6	–	–	–	–	69	–	95,97
	10	–	–	–	–	100	–	96
30.9-31.9	2	0	0	0	97	70,91	100	73,95
	4	0	0	0	100	89	96	95,96
	6	0	0	0	97	89	100	93,100
	10	0	0[a]	0[a]	93	94	–	100
32.5-32.9	2	–	–	–	–	41,100	94	100
	4	–	–	–	–	61,100	100	100
	6	–	–	–	–	24,100	96	93
	10	–	–	–	–	19,42	–	91
33.2-33.5	2	–	–	–	–	83	88	100
	4	–	–	–	–	86	31	100
	6	–	–	–	–	25	20	48
	10	–	–	–	–	8	–	–
33.6-33.8	2	–	–	–	–	100	39	96
	4	–	–	–	–	100	18	55
	6	–	–	–	–	4	3	24
	10	–	–	–	–	0	–	–
34.3	2	–	–	–	–	–	68	88
	4	–	–	–	–	–	36	33
	6	–	–	–	–	–	15	4
	10	–	–	–	–	–	–	–
34.6-34.7	2	–	–	–	–	–	22	92
	4	–	–	–	–	–	0	13
	6	–	–	–	–	–	0	0
	10	–	–	–	–	–	–	–

[a]Duration in these experiments, 12 hours.
[b]Duration in these experiments, 15 hours.

crease thereafter. The total increase in tolerance for two hours of exposure is approximately 9.0°, or 10.6° above base level. For 10 hours' exposure, the increase over base level is about 8.6°.

Rana sp. (*pipiens* group)
Table 2, Figure 1

The form used in these experiments is that unnamed[1] species of the *pipiens* group referred to in the recent literature as the "southern" form (Mecham, 1968; Pace, 1974; Platz and Platz, 1973) and by me as *Rana pipiens* (Zweifel, 1968). The species inhabits streams and canyon pools in the Chiricahua Mountains and other ranges of east-central and southern Arizona. All my experiments utilized eggs collected in the field in the Chiricahua Mountains, either on the grounds of the Southwestern Research Station, elevation 5400 feet (1650 m.), or in the vicinity of Herb Martyr Dam, 2 miles upstream in the same (Cave Creek) drainage at 5600 feet (1700 m.). There is no possibility of confusion of the identity of the egg masses, as this is the only species of *Rana* known at these localities, and no other anuran with even remotely similar egg masses occurs there.

The maximum constant temperature that the youngest embryos can tolerate is approximately 31.5° (Zweifel, 1968). In the present experiment, I conducted no short-term tolerance tests during the early embryonic stages. Embryos in stages 6 and 7 tolerated 35.0° for six hours. In view of the experiments on stage 8, probably they were within one degree or less of their upper limit. A change in tolerance is evident in the data for stage 8 and 9 embryos: stage 8 embryos were killed after two hours at 36.4°, whereas stage 9 embryos showed high survival at 36.2° for two hours and marginal survival for four hours at 36.3°. The upper limit for stage 9 was not determined precisely, but it is below 37.6° for two hours. Embryos in stage 12 marginally tolerated 37.4° for two hours and 37.0° for six hours; tolerance of stage 13 embryos for two hours at 37.4° is improved. There is little increase in tolerance once the embryo has completed gastrulation. The two-hour tolerance figures for embryos as late as stage 17 are no higher (37.5°) than for stages 13-15, although the six-hour tolerance improves to match the two-hour tolerance level. Experiments run on stage 16L-17 embryos at 38.3-38.5° produced highly variable results, as might be expected in the region of the limiting temperature. In six experiments, the range of survival was from zero to 69 percent, mean 21.6 percent. Probably the two-hour limiting temperature is about 37.8-38.0°.

Following a relatively rapid increase in tolerance during early cleavage stages, the rate of increase slacks off and does not increase greatly during gastrulation. The total increase by the completion of gastrulation is only about 6.1° above the base level of 31.5° and probably does not exceed 6.5° in all.

Scaphiopus bombifrons Cope
Table 3, Figure 2

Parental frogs used in these experiments came from Animas Valley, Hidalgo County, New Mexico, and from San Simon Valley in Hidalgo County and Cochise County, Arizona. Voucher specimens are AMNH numbers 90220-90225 and 91984-91990. Frogs of this region have a mating call markedly different from that of *S. bombifrons* in the eastern part of its range (Bogert, 1960; Pierce, 1976), and it is likely that the eastern and western populations currently referred to this name belong to different species. It is important for future workers to keep this in mind when doing comparative studies. An additional complication is that in my study area *S. bombifrons* and *S. multiplicatus* occasionally hybridize. I used only frogs that showed the distinctive morphological traits of *bombifrons*. Embryos used in the experiments came both from eggs deposited and fertilized by amplexing pairs in the laboratory and from eggs obtained through induced ovulation and artificially inseminated.

The upper limiting temperature for stage 3 under continuous exposure is approximately 32.5° (Zweifel, 1968). In stage 7 there was marginal survival for two to 10 hours of exposure to 36.3°, and 100 percent mortality occurred at

[1] A formal description of this species by Platz and Mecham is in manuscript.

TABLE 2
Rana sp.: **Percentage of Normal Development After Exposure for Time Shown in Column 2 When in Stages Shown in Columns 3 to 11**

Temp. °C.	Hours Exposed	St. 6,7	St. 8	St. 9	St. 12	St. 13	St. 14	St. 15L	St. 16L-17	St. 18-19
34.7-35.3	2	96,100	–	73	–	96	–	–	96	–
	4	86,95	–	84	100	100	–	–	91	–
	6	52,73	–	93	–	100	–	–	97	–
	10	–	–	94	100	82	–	–	42	–
36.0-36.5	2	–	0,0	93	58,100	84,95	100	100,100	–	–
	4	–	0	45	88,90	100,100	100	92	–	–
	6	–	0	0	88	88,100	100	96	–	–
	10	–	0,0	0	44	34,74	100	100	–	–
36.6-37.0	2	–	–	–	96	–	–	–	100,100	–
	4	–	–	–	87	–	–	100	97,100	–
	6	–	–	–	77	–	–	93	90,100	–
	10	–	–	–	7	–	–	85	45,93	–
37.3-37.7	2	0	–	0	31,70	85,100	100	88,100	93,100	–
	4	0	–	0	0,0	0,20	96	64,92	86	–
	6	0	–	0	0	0,23	84	0,88	23,100	–
	10	–	–	0	0	0,0	–	0,9	0	–
38.1-38.5	2	–	–	0	0,0	0,0	0,2	0	21.2(0-69,6)	0
	4	–	–	0	0,0	0,0	–	0	0,0	0
	6	–	–	0	0	0,0	0	0	0,0,0	–
	10	–	–	0	0	0,0	0	0	0	0
38.6-38.8	2	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	0,0	–
	6	–	–	–	–	–	–	–	0,0,0	–
	10	–	–	–	–	–	–	–	0,0,0	–
39.0-39.7	2	–	–	0	0	0,0	–	0,0	0(0-0,4)	–
	4	–	–	0	0	0,0	–	0,0	0,0,0	–
	6	–	–	0	–	0,0	–	0,0	0(0-0,4)	–
	10	–	–	0	–	0,0	–	0,0	0,0,0	–

37.5°. In contrast to the situation in stage 7, embryos at slightly later stages–late stage 8 and early stage 9–showed a high percentage of survival for up to six hours at 37.5° and for two hours at 37.9°. Marginal results obtained at longer durations at the latter temperature, and temperatures of 39.1° and higher were invariably fatal. Embryos in stage 12 survived well for two hours, marginally for four hours at 39.5°, and marginally for two hours at 39.9°. No embryos were exposed for longer periods. The only two experiments on stage 13 embryos showed no survival after 10 hours at 40.0° and marginal survival for the same period at 39.2°. Stage 17 embryos showed a slight increase in tolerance over those in stage 12 in that 100 percent in one experiment survived two hours at 39.7°, but six hours at 40.0° and four hours at 40.5° were not tolerated.

A rapid increase in tolerance takes place between early and late cleavage stages (*ca.* 2° between stage 7 and middle stage 8) and a relatively high rate evidently continues to the beginning of stage 12. However, the absence of data leaves it uncertain whether the later increase in tolerance takes place evenly or is largely restricted to the early period of gastrulation. Probably the maximum level of tolerance is about 40.0° and is attained by stage 13, in view of the marginal survival of embryos in stage 12 at 39.9°. It is unlikely that the maximum tolerance exceeds 40.0° for two hours, in light of the poor performance

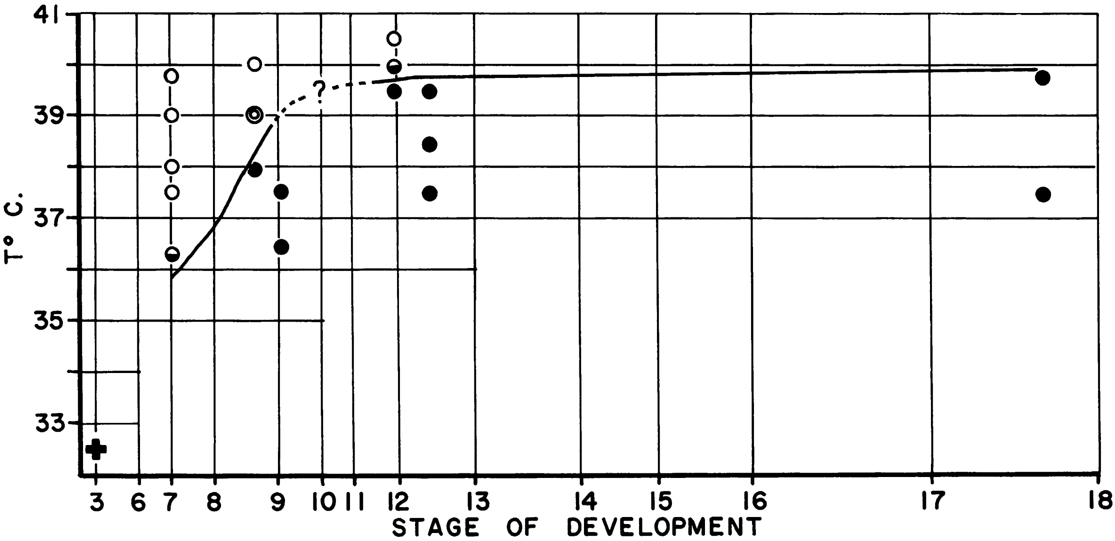

FIG. 2. Ontogenetic change in two-hour temperature tolerance in embryos of *Scaphiopus bombifrons*. See figure 1 for mode of presentation.

of stage 17 embryos for longer periods at 40.0° and 40.5°.

Scaphiopus couchii Baird
Table 4, Figure 3

Material for these experiments was collected in Animas Valley and in San Simon Valley, Hidalgo County, New Mexico. Parental voucher specimens are AMNH numbers 87981, 87982, 90229, 90230, and 91991-91994. In two experiments eggs collected in the field were used. (Eggs of this species are readily distinguished from those of sympatric forms: Zweifel, 1968, p. 54.) In all other experiments eggs were oviposited and fertilized by amplexing pairs in the laboratory. *Scaphiopus couchii* occurs widely in desert and semi-desert regions of the Southwest, and in subtropical scrub in western Mexico.

The base-level temperature is approximately 34.0° (Zweifel, 1968). There was a high percentage of survival up to 10 hours at 36.5° in stage 6, but survival was marginal for two hours at 37.0° and stage 7 embryos failed completely at 37.1°. The results of experiments in late cleavage stages (8 and 9) were more variable than at other stages. Experiments in early stage 8 gave good survival for two hours at 36.2° but little normal development at 37.7° and higher. An experiment in middle stage 8 gave marginal survival at 37.5° and poor survival at 38.5°. Experiments in late stage 8 gave good survival as high as 39.7° but greatly reduced viability at 39.9°. So far the results are consistent with a rapid increase in temperature tolerance during stage 8. However, the only experiment run on embryos in early stage 9 gave marginal survival (40 percent) as low as 36.3° and no normal development at all at 37.5°, 38.4° and 39.3°. It is possible that in this instance some detrimental factor other than temperature may have been operative. This is indicated because embryos of the same clutch showed 90-100 percent viability at 36.4° over two to four hours in an experiment initiated at stage 7. Also, stage 16 embryos from the same clutch did well at higher temperatures. Thus, I have ignored this experiment in drawing the tolerance curve (fig. 3), but have entered the data in that figure and in table 4. By middle stage 12 embryos easily tolerate 40.1° for two hours. The maximum temperature tolerated for two hours evidently is less than 40.6° (almost no normal development in stage 16L) but is at least 40.2° (90 percent viability in stage 17E). Two

TABLE 3
Scaphiopus bombifrons: Percentage of Normal Development After Exposure for Hours Shown in Column 2 When in Stages Shown in Columns 3 to 7

Temp. °C.	Hours Exposed	St. 7	St. 8L-9E	St. 12	St. 13	St. 17
36.3-36.4	2	45	93	–	–	–
	4	56	82	–	–	–
	6	20	87	–	–	–
	10	37	–	–	–	–
37.4-37.6	2	0	79	100	–	–
	4	0	100	100	–	100
	6	0	88	–	–	–
	10	0	–	–	–	–
37.9-38.4	2	0	73	93	–	–
	4	0	31	88	–	–
	6	0	39,81	–	–	–
	10	–	–	–	–	–
39.0-39.5	2	0	0,0	91,100	–	–
	4	0	0	56	–	–
	6	0	0	–	–	–
	10	0	–	–	60	–
39.7-40.0	2	0	0	52	–	100
	4	0	0	–	–	–
	6	0	0	–	–	8
	10	0	–	–	0	–
40.5-40.6	2	–	–	12	–	–
	4	–	–	–	–	0
	6	–	–	–	–	–
	10	–	–	–	–	–

experiments in stage 16L and 17 at 40.3° produced somewhat marginal (but far from poor) results–60 and 76 percent normal development.

The amount of ontogenetic change in temperature tolerance of *S. couchii* embryos is not impressive (*ca.* 6.3° over its high base level), but the apparent rapidity of change in late cleavage stages and the early attainment of a high degree of resistance are noteworthy.

Scaphiopus multiplicatus Cope

I conducted few experiments on this species, which was studied intensively by Brown (1967a), who used material from the same region as I (vicinity of Rodeo, Hidalgo County, New Mexico). As formerly defined, *S. hammondii* had a disjunct distribution: semi-arid (but not desert) areas of California and Baja California, and a large, plains and desert area of the Southwest centered on New Mexico. Brown recently (1976) has shown that these disjunct populations would better be considered distinct species, with the name *multiplicatus* used for the species I studied.

Brown (1967b) and Zweifel (1968) independently determined the limiting temperature (base level) at 32.5°. Brown's (1967a) data for short-term tolerances are not exactly comparable with mine for this and other species, as he pooled his data into "Early cleavage 3-5," "Late cleavage 7-9," and "Gastrula 10-12" (Brown, 1967a, table 2). Thus, any changes in tolerance that may have occurred within the developmental span of one of his groups (such as the change in stages 7 and 8 that I found in *S. bombifrons*) would not be apparent.

Embryos that I exposed to 36.4° at stage 9E showed a high rate of survival for as long as 10 hours but other groups suffered virtually 100 percent fatality in as little as two hours at 37.6°,

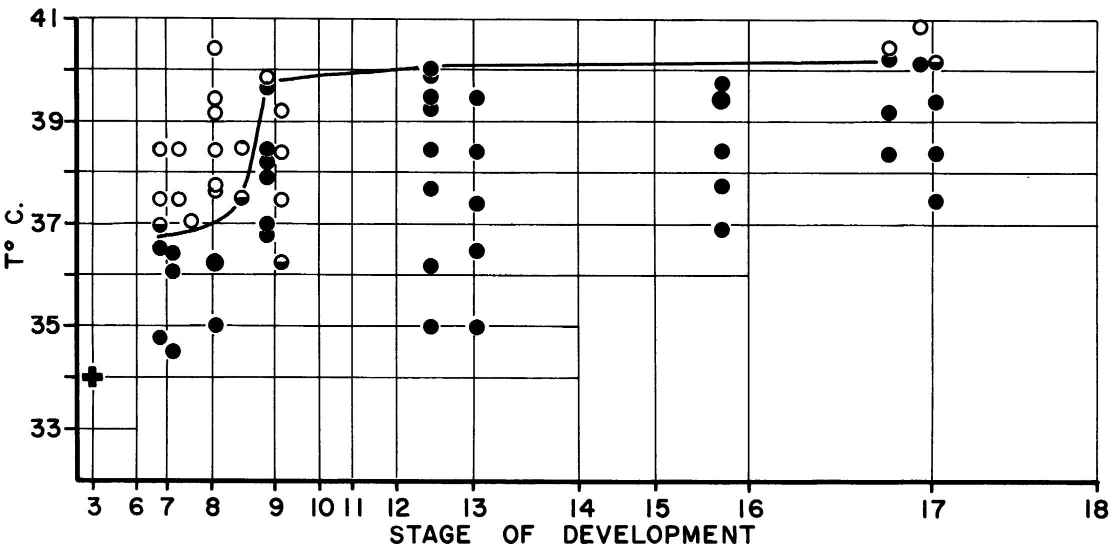

FIG. 3. Ontogenetic change in two-hour temperature tolerance in embryos of *Scaphiopus couchii*. See figure 1 for mode of presentation.

38.4°, and 39.3°. In contrast, all embryos from the same clutch exposed to 39.2° at stage 10 for two and six hours survived. Thus, there appears to be a rapid increase in tolerance at the commencement of gastrulation. Brown (1967a) found 90 to 100 percent survival of gastrulae exposed to 40.0° for two hours, but embryos in stage 20 did not survive three, four, and six hours at 40.4°. However, stage 16 embryos in my experiments survived 40.4° for two hours (90%) but did much less well at longer periods (35% at four hours, 0% at six hours). The upper limiting temperature for two-hour exposure may be set at 40.4°, slightly higher than the 39.0° or 40.0° of Brown's estimate.

Bufo cognatus Say
Table 5, Figure 4

This species of *Bufo* is distributed widely over the plains of central North America and in all but the driest deserts of the Southwest. Eggs used in my experiments came from two toads. Ovulation was induced by implantation of homospecific pituitary glands; artificial fertilization was employed. Voucher specimens are AMNH 90210 and 90211 from San Simon Valley, Hidalgo County, New Mexico, and AMNH 90212 and 90213 from the vicinity of Animas, Hidalgo County, New Mexico.

The estimated base-level temperature is 33.5° (Zweifel, 1968). Embryos in stage 6 tolerated 36.0° for 10 hours, but there was total mortality at 38.3.° in two hours. Embryos in middle stage 8 tolerated 38.4° for 10 hours, whereas others in early stage 8 showed no survival for two hours or longer at 39.5°. Tolerance of gastrulae (early stage 12) extends to at least 40.4° for four hours. No six-hour or 10-hour tests were run on this stage at higher than 39.6°, and no two-hour or four-hour tests at higher than 40.4°. Tolerance late in stage 16 reaches at least 40.5° for six hours, but there were no experiments at higher temperatures or for longer durations.

It is unfortunate that material was insufficient to permit more experiments and more exact determination of the tolerance of *Bufo cognatus*, for this species clearly is the most heat-resistant of any studied. A curious aspect of the data on this species is that there is no evidence of a graded effect of duration of exposure on survival. That is, in all instances where embryos in a given experiment were tested over different time periods, the result was essentially all or none; either

TABLE 4
Scaphiopus couchii: Percentage of Normal Development After Exposure for Hours Shown in Column 2 When in Stages Shown in Columns 3 to 8

Temp. °C.	Hours Exposed	St. 6,7	St. 8,9	St. 12	St. 13	St. 15L	St. 16L,17
34.5-35.0	2	83,100	71	90	100	–	–
	4	86,100	100	93	100	–	–
	6	91,100	76	94	77	–	–
	10	88,93	93	82	–	–	–
36.0-36.7	2	77,90,100	40,60,81	89	85	–	–
	4	58,100,100	60,80	100	73	–	–
	6	66,91	90,100	94	95	–	–
	10	70,92	90,90	100	–	–	–
36.8-37.2	2	0,41	79,100	–	–	100	–
	4	0,21	65,100	–	–	96	–
	6	0,17	77,90	–	–	96	–
	10	2,9	50,100	–	–	90	–
37.5-37.9	2	0,0	38 (0-94,5)	79	95	100	100
	4	0,0	32 (0-86,4)	93	100	97	100
	6	0	53 (8-90,4)	83	82	96	93
	10	0	0,34,94	100	–	95	100
38.3-38.6	2	0,10,0	25 (0-88,4)	89	100	88	87
	4	0,8,0	0,13,75	87	100	95	83,100
	6	0,2	14,46,100	100	85	100	100
	10	0	0,100	92	–	100	96,100
39.2-39.7	2	–	22 (0-88,4)	75,100	100	100,100	87
	4	–	0,0,87	77,81	100	87,94	90,100
	6	–	0,0,68	65,77	83	81,100	93
	10	–	0,0,35	78	–	75	68,82
39.8-40.4	2	–	0,27	71,100	–	100	60,76,90
	4	–	0,45	48	–	89	52,54,61
	6	–	0,10	30	–	69	0,20
	10	–	0,0	0	–	–	0,34
40.6-40.9	2	–	–	–	–	–	0,9
	4	–	–	–	–	–	0,0
	6	–	–	–	–	–	0
	10	–	–	–	–	–	–

there was no survival, or there was a high survival percentage at all time periods. Such a result might be expected where there is a relatively great difference between two experimental temperatures, as between 36.0° (91% survival for 10 hours) and 38.3° (no survival in two hours). But in experiments at stage 8 conducted at 38.4° and 39.5°, viability dropped from 100 percent at 10 hours to no survival at two hours.

There are too few data points to establish precisely the shape of the tolerance curve. Clearly, a considerable increase in tolerance takes place between stage 6 and middle stage 8 and between stages 8 and 12, but whether the rate of increase slows down in late cleavage and then increases again during gastrulation was not determined. The total increase in tolerance is at least 7° over the base level of 33.5° and may well extend one-half a degree higher or even more.

Gastrophryne olivacea (Hallowell)

Through the courtesy of Dr. Charles H. Lowe and Mr. D. Frost I received a small number of eggs of this species (from two different clutches) collected at Tracy's Trading Post, 97 miles west

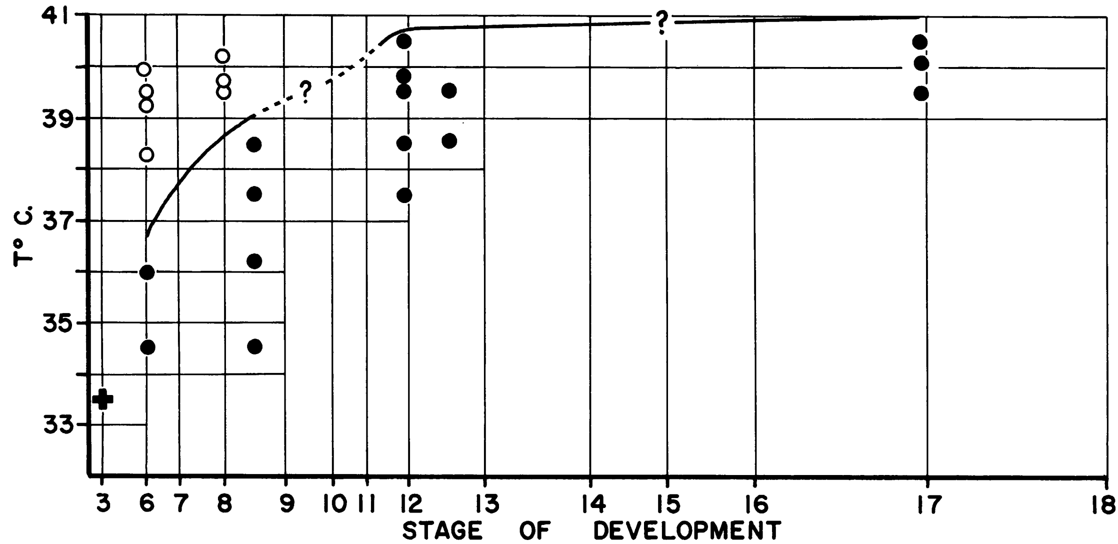

FIG. 4. Ontogenetic change in two-hour temperature tolerance in embryos of *Bufo cognatus*. See figure 1 for mode of presentation.

of Tucson, Pima County, Arizona. Identification was made on the larvae that developed from the eggs. They had a characteristic microhylid morphology, and *G. olivacea* is the only species of microhylid known in Arizona. The Arizona population represents the northern part of a geographic range that includes much of the Great Plains and extends westward across northern Mexico.

Ballinger and McKinney (1966) reported preliminary experiments on constant-temperature tolerances of embryos of this species: "... our experiments show larvae being obtained at 32.6° and a single individual hatched at 38.9°; although all individuals failed to hatch at 34.1°." The numbers of eggs used and percentage of viability are not given, and it is not clear whether only the youngest embryos were used, as the authors merely refer to "eggs in early cleavage." Hence, some of the variability may have resulted from differences in ages of embryos at the initiation of the experiments. About all that can be said is that the base-level temperature probably is below 34.0°.

Material available to me was sufficient only for experiments in two stages (14E and 17E) for two- and 10-hour runs at four temperatures. Embryos in stage 14E experienced a high percentage of survival (91-100%) for two hours at 36.3°, 37.0°, 38.8°, and 39.3°. Ten-hour experiments were similarly successful (93-97%) at 36.4° and 37.0°, although there was only 38 percent survival at 39.1° and zero viability at 39.6°. Embryos exposed initially in stage 17E were more resistant over the longer period of time. The lowest viability recorded was 77 percent for 10 hours at 39.3°.

The high percentage of survival for 10 hours at 39.3° indicates that *Gastrophryne olivacea* may well equal or even exceed the species of *Scaphiopus*, which have upper tolerance limits no more than one degree above that easily tolerated by *G. olivacea*. In this connection, it is worth noting that *G. olivacea* probably has its lower limiting temperature at about 19 or 20 degrees (Ballinger and McKinney, 1966), which is higher than that of any of several southwestern anurans previously studied (Zweifel, 1968) and is indicative of a relatively high range of temperature adaptation.

Hyla regilla Baird and Girard

I did no work on this species but summarize the available information for comparative purposes. *Hyla regilla* ranges along the Pacific Coast

TABLE 5
Bufo cognatus: **Percentage of Normal Development After Exposure for Hours Shown in Column 2 When in Stages Shown in Columns 3 to 6**

Temp. °C.	Hours Exposed	St. 6	St. 8	St. 12	St. 16L
34.5-34.6	2	100	98	–	–
	4	82	98	–	–
	6	98	100	–	–
	10	98	97	–	–
36.0-36.2	2	100	100	–	–
	4	98	98	–	–
	6	96	100	–	–
	10	91	93	–	–
37.4-37.5	2	–	100	98	–
	4	–	92	98	–
	6	–	93	–	–
	10	–	98	–	–
38.2-38.6	2	0	86	96,100	–
	4	0	–	98,100	–
	6	0	93	98	–
	10	–	100	93	–
39.3-39.7	2	0,0	0,0	95,100	80
	4	0,0	0,0	73,79	–
	6	0,0	0,0	92	–
	10	–	–	84	–
39.8-40.2	2	0	0	63	95
	4	0	0	70	95
	6	0	0	–	85
	10	–	–	–	–
40.4-40.5	2	–	–	47	95
	4	–	–	95	80
	6	–	–	–	86
	10	–	–	–	–

from Canada to the tip of Baja California and occurs virtually wherever there is fresh water, from deserts to temperate rain forest, and from sea level to high in the mountains.

In a careful study, Brown (1975) established that stage 3 embryos from coastal southern California had an upper limiting temperature of 29.6° for constant exposure, whereas this limit was 28.0° for embryos from western Washington.

Schechtman and Olson (1941) conducted a pioneer study of the tolerance of embryos to short-term exposure to extremely high and low temperatures. They used embryos of *Hyla regilla* from coastal southern California, and it is reasonable to assume that the population is similar if not identical in its limiting temperatures to that studied by Brown. Schechtman and Olson used methods quite comparable with those adopted by Herbert A. Brown (1967a) and by me: they exposed embryos in blastula stage (possibly both stages 8 and 9), gastrula stage (possibly all three stages 10-12), neurula stage (stages 14 and 15?), tail bud stage (probably stage 17), and "tail fin, prehatching" (stage 19?) to elevated temperatures in single degree steps from 29.0° to 39.0° for periods of two to six hours. Results were recorded as percent viability. Not all stages were run at all time and temperature combinations.

The results of these experiments were fairly clear-cut. Blastulae survived well (98%) for two hours at 34.0°, marginally for two to five and one-half hours at 35.0° (33-58%), and not at all at 37.0° (no experiments were run at 36.0°). Gastrulae exhibited greater tolerance, with 92-

100 percent survival over two to four hours at 35.0°, 88 percent survival for two hours at 36.0°, and only marginal tolerance (56%) for two hours at 37.0°. Temperatures of 38.0° and 39.0° were not tolerated at all. Neurulae were tested at only two critical temperatures: 38.0° (40% survival over two hours) and 39.0° (0% over two hours). Tail bud and "tail fin" embryos did better than neurulae, with 80 percent and 100 percent viability for two hours at 38.0°, but neither tolerated 39.0°.

The data of Schechtman and Olson are not sufficiently refined to permit the shape of the tolerance curve to be plotted accurately. There is clearly an increase in two-hour tolerance from about 34.5° in "blastula" to about 36.5° in "gastrula," but whether this increase takes place gradually over development from early stage 8 to late stage 12 or is confined to a shorter part of the developmental period cannot be said. In any event, the attainment of maximum tolerance is somewhat prolonged, as the tolerance level of gastrulae is only slightly more than 80 percent of the final level, compared with more than 90 percent in all species I studied except *Rana sylvatica.*

DISCUSSION

A species copes simultaneously with problems posed by temperature of the breeding habitat in two ways. On one level is behavioral adjustment, the restriction of oviposition to times and sites where detrimental temperatures are unlikely to be encountered. On another level are the physiological responses of the embryo to temperature. If at the first level the species is sufficiently conservative in its times and places of breeding (or if the habitat is unusually stenothermic), then the need for broad embryonic adaptation is obviated. But such conservativeness penalizes a species by greatly restricting its opportunities for breeding. At the other extreme there appear to be physiological limitations that prevent early embryos from being simultaneously adapted to both extremely cold and extremely warm conditions. Early embryos of different species may differ greatly in their range of temperature tolerance, and no species can take advantage of the total range of conditions exploited by frogs as a whole.

Given a restricted range of temperature tolerance at the beginning of life and having no way of actively avoiding detrimental temperatures should they occur, the obvious adaptive change is for the embryo to widen its range of tolerance as it grows. Some degree of broadening of temperature tolerance probably is characteristic of anuran embryos generally. Atlas (1935, p. 298) suggested that, in early embryos of *Rana pipiens* (in the restricted taxonomic sense), decrease in susceptibility to injury by elevated temperatures "is approximately proportional to the number of cleavages. This would be in harmony with a gradual conversion of yolk to cytoplasm as the potent factor in the increased tolerance." His experimental technique involved exposing embryos in each of the first six cleavages to 32.5° for various lengths of time to determine the duration necessary to produce nothing but abnormal embryos. The temperature he used is 4.5° above the base level for *pipiens* (28.0°; Moore, 1949) and almost certainly within the tolerance of later embryos.

Svinkin (1962) essentially studied the same phenomenon in *Rana temporaria* with a somewhat different method. He exposed embryos of successive cleavage generations to 36.0°, noting how many minutes of exposure it took to suppress completely any sign of additional cleavage. He detected no increase in resistance to this temperature until the sixth cleavage generation (stage 7) and indicated that change in heat resistance is related to the synthesis of new proteins beginning in the blastula stage. I suspect that the failure of Svinkin's experiments to reveal change in heat resistance earlier in development is related to the rather high temperature employed, which is 11.0° above the base level of *temporaria* (25.0°; Moore, 1951). In fact, I question whether embryos of *temporaria* could develop normally at all if exposed to 36.0° for any length of time. This species is closely related to *Rana sylvatica* and has similar upper and lower base levels. Note that the maximum two-hour tolerance of *sylvatica* is 34.6°.

So if an ontogenetic increase in resistance to elevated temperatures is an inherent capacity of the anuran embryo, adaptive changes acting to increase tolerance for higher temperatures might be expected to act in one or both of two ways:

by an increase in the total magnitude of the ontogenetic change; by increasing the rate of change, establishing a high level of tolerance earlier in development.

AMOUNT OF CHANGE IN TEMPERATURE TOLERANCE

The extent of change in temperature tolerance may conveniently be expressed as the difference between the base-level tolerance and the maximum tolerated over the period of exposure (2, 4, 6, or 10 hours). It would perhaps be better to calculate the amount of change in relation to the tolerance of the stage 3 embryos for the particular period of exposure, rather than relative to the base-level constant temperature criterion, but the appropriate datum is available for only one species, *Rana sylvatica*. Pertinent data are assembled in table 6 and summarized in figure 5.

Present information suggests that the upper limit of temperature tolerance reached by any anuran embryo (for two hours of exposure) is in the vicinity of 41.0°. Thus, an upper limit is set to the maximum change possible, and the higher the upper base level with which the embryo com-

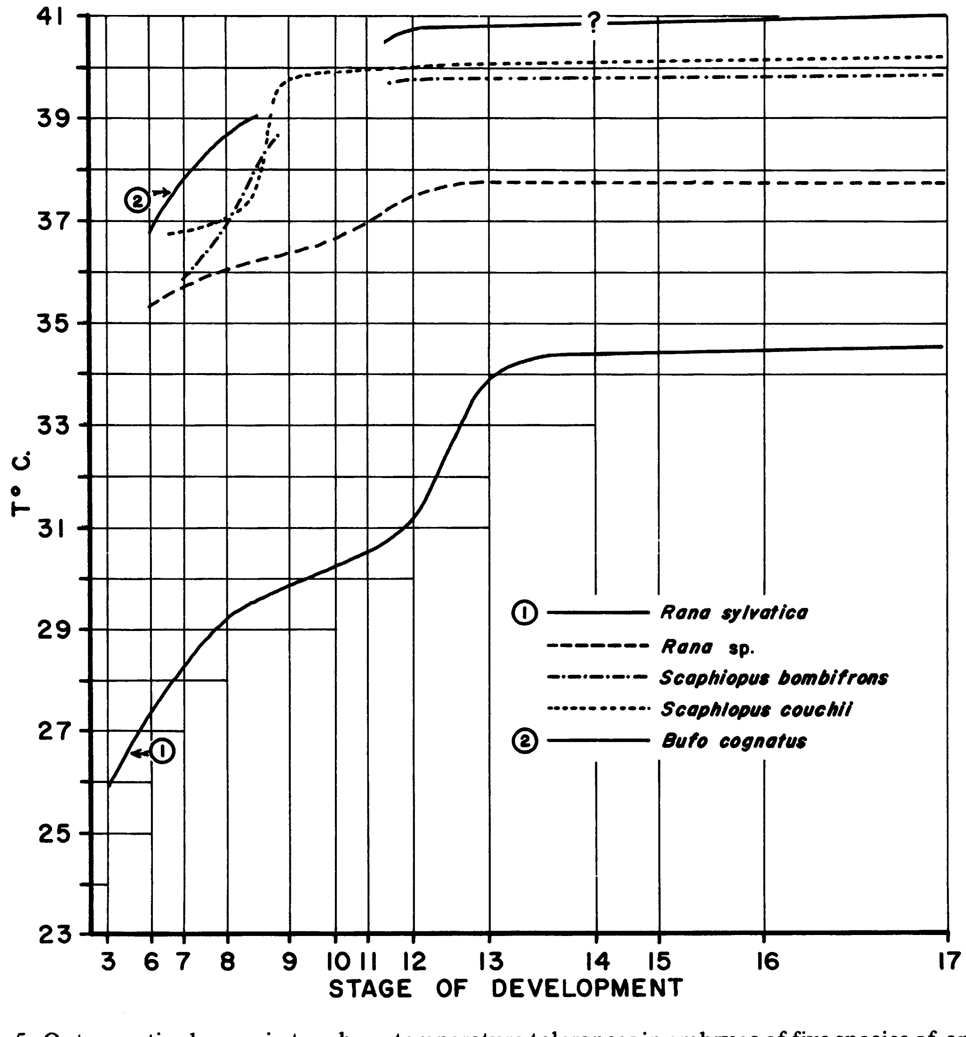

FIG. 5. Ontogenetic changes in two-hour temperature tolerances in embryos of five species of anurans.

TABLE 6
Maximum Temperatures Tolerated by Anuran Embryos for Different Periods of Exposure[a]

Species	Base Level	2 Hours	4 Hours	6 Hours	10 Hours
R. sylvatica	24.0°	34.6° (10.6°)	33.7° (9.7°)	32.9° (8.9°)	32.6° (8.6°)
H. regilla[b]	29.6°	38.0° (8.4°)	–	–	–
R. sp.	31.5°	37.7° (6.2°)	37.6° (6.1°)	37.5° (6.0°)	36.8° (5.3°)
S. bombifrons	32.5°	40.0° (7.5°)	39.2° (6.7°)	39.2° (6.7°)	39.2° (6.7°)
S. multiplicatus[c]	32.5°	40.4° (7.9°)	40.0° (7.5°)	39.0° (6.5°)	–
S. couchii	34.0°	40.3° (6.3°)	39.8° (5.8°)	39.8° (5.8°)	39.5° (5.5°)
B. cognatus	33.5°	>40.5° (>7.0°)	>40.5° (>7.0°)	>40.5° (>7.0°)	–

[a]Temperature in degrees Celsius; figures in parentheses represent increase over base-level tolerance.
[b]Data from Brown (1975, base level) and Schechtman and Olson (1941, two-hour tolerance).
[c]Base-level datum from Brown (1967b) and Zweifel (1968); other data from Brown (1967a) and the present work.

mences life, the less change is possible. Species with relatively high base levels (e.g., *Scaphiopus couchii*, 34.0°; *Bufo cognatus*, 33.5°) would necessarily be limited to about six to seven degrees of increase. The lower the base level, the more increase could theoretically take place, and we see *Rana sylvatica* (base level 24.0°) and *Hyla regilla* (base level 29.6°) with the greatest absolute increases in two-hour tolerance (10.6° and 8.4°, respectively). Among the species for which we have adequate data, those most likely to be exposed to high temperatures–*Bufo cognatus* and the three species of *Scaphiopus*–all have pushed the maximum tolerance to 40.0° or higher. The two *Rana* and *Hyla regilla*, breeding in places where or at times when extremely high water temperatures do not occur, do not so closely approach the hypothetical maximum of 41.0°.

There is another way of looking at the extent to which adaptation to high temperature has taken place by the route of increasing maximum tolerance. If we assume that the maximum temperature any embryos can stand for two hours' exposure is 41.0°, the difference between that figure and base-level tolerance for a given species (the maximum possible increase) is divided into the observed increase, giving the percentage of possible increase actually realized. For the seven species tabulated in table 6 these are:

Rana sylvatica, 62.3%
Hyla regilla, 73.7%
Rana sp., 65.3%
Scaphiopus bombifrons, 88.2%
Scaphiopus multiplicatus, 92.9%
Scaphiopus couchii, 90.0%
Bufo cognatus, 93.3%

It is clear that the last four species have done about as much as is possible, and the slight differences among them might be attributed to experimental error. The gulf between these species and the two *Rana* is significant, as is the lesser difference between the *Rana* and the *Hyla*.

It might be assumed a priori that a longer period of exposure to elevated temperatures would be less well tolerated than a shorter one. This is clearly the case in the two species of *Rana* and in the three species of *Scaphiopus*. In these species the maximum temperature tolerated for 10 hours of exposure is about one to two degrees lower than the two-hour tolerance, and in individual experiments conducted at marginal temperatures there is usually a gradation in percentage of embryos surviving from short- to long-term exposure (tables 1-4). Quite in contrast, no such effects are apparent in the data for *Bufo cognatus* (table 5). If the embryos of *Bufo* are viable at all, they seem to survive as well at six or 10 hours as for two hours. Although embryos were run at as high as 40.5°, the limiting temperature was not reached. Thus, it is possible that at even higher temperatures a difference between long-term and short-term tolerances might appear. However, there is no evidence for this where limiting temperatures were attained in cleavage stages.

RATE OF CHANGE IN TOLERANCE DURING ONTOGENY

The shape of the curve that describes the temperature tolerance at different points during development cannot be accurately determined for all species studied. All show the initial increase in tolerance anticipated from the work of Atlas (1935) and all have attained their maximum tolerance or close to it by stage 13, when gastrulation is completed (fig. 6). The critical period where advancement of high tolerance to an earlier stage may take place includes the blastula (stage 8) to yolk plug (stage 12) periods in development.

Data for the two species of *Rana* are good, and it is clear that *Rana* sp. attains a near-maximal level slightly earlier than *Rana sylvatica*—at the beginning of stage 12 rather than at the beginning of stage 13. *Scaphiopus couchii* reaches a high level of tolerance quite early in development, by the end of stage 8. *Scaphiopus bombifrons* and *Bufo cognatus* lack data for the critical stages 9 and 10. It is clear from these data, imperfect though they are, that the attainment of maximum or near-maximum tolerance is not immutably tied to a particular stage of development. A slight though significant difference is seen between two species of *Rana*, and *Scaphiopus couchii* adapts markedly earlier.

Hyla regilla appears to have a more gradual development of tolerance than the other species, for only about 76 percent of the adaptation has been accomplished by gastrula stage, and some improvement in tolerance is registered as late as between neurula and tail-bud stages. The shape of the curve probably is more like that of *Rana sylvatica* than any other species studied.

TEMPERATURE TOLERANCE AND RATE OF DEVELOPMENT

One other means of adapting to high temperature needs to be mentioned. Assuming that an ontogenetic increase in tolerance is going to take place, whether modified in scope and precocity or both, if one embryo develops more rapidly than another at a given temperature, the first will achieve a higher degree of tolerance earlier than the second. Thus, a rapid rate of embryonic development, commonly thought of as an adaptation to an ephemeral breeding site, functions as well to shorten the period in which an embryo is most sensitive to high temperature (Brown, 1967a; Zweifel, 1968).

TEMPERATURE TOLERANCES IN RELATION TO CONDITIONS IN BREEDING SITES

Rana sylvatica, which ranges from Alaska to Georgia, is one of the most widely distributed frogs in North America. Wherever it occurs it is commonly the anuran that breeds earliest in the year. I know the species best in the vicinity of New York City, where breeding is typically initiated in middle to late March. This is a time of highly variable and unpredictable weather conditions, when embryos could easily be exposed to temperatures as low as freezing to 20° or higher within the span of less than a day.

The eggs typically are deposited in water of a rather low temperature. On several occasions I have found frogs in amplexus or fresh egg masses (no later than stage 3) in water at 6.5° to 11.0°. But high temperatures approaching those lethal for young embryos must occasionally be reached. Once I found a mass freshly deposited (jelly still unexpanded, ova unrotated) at noon in water at 17.2°. Three hours later on this sunny afternoon the temperature in the middle of the mass was 22.5° (adjacent water 20.4°). Given a slightly warmer day and a mass somewhat closer to the surface (the depth at the middle of this mass was about 10 cm.), it is conceivable that the two-hour lethal temperature of about 26.0° for stage 3 might be reached. However, any such danger to the embryos rapidly passes. Even at as low as 10.0°, *Rana sylvatica* embryos reach stage 8 in 24 hours after first cleavage (about 27 hours after fertilization; Moore, 1939), and by this time their two-hour tolerance exceeds 29.0° (fig. 1). I think that water temperatures high enough to injure early embryos of *Rana sylvatica* occur in the breeding season only as extremely rare events. John A. Moore (personal commun.) witnessed such an event at Alpine, New Jersey, when unseasonable and prolonged high temperatures evidently were responsible for the death of an entire year class of *sylvatica* embryos. However, high temperature acting together with reduced oxygen tension is a source of danger to

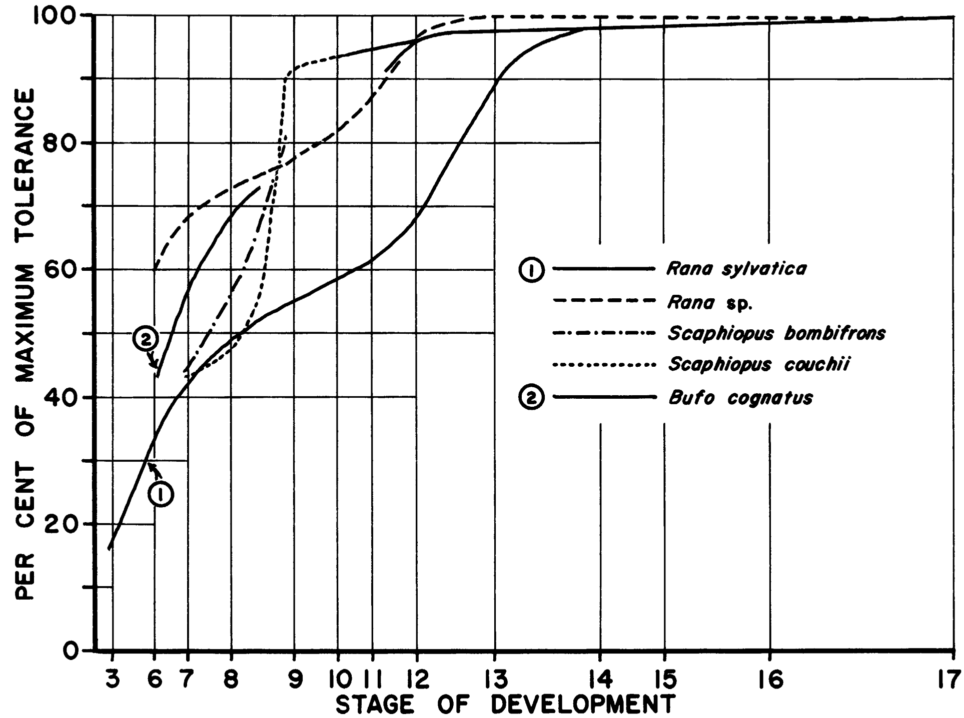

FIG. 6. Ontogenetic changes in two-hour temperature tolerances in embryos of five species of anurans expressed as percentage of maximum tolerance attained by each species.

later embryos, especially those in the center of the globular egg mass (Moore, 1940).

Rana sp. deposits its eggs in streams, both in pools and in running water, in mountain canyons as well as in "tanks" (cattle watering ponds) at lower elevations. In situations where I have found egg masses in the Chiricahua Mountains (Zweifel, 1968, and subsequent observations) it is unlikely that high water temperatures pose a serious danger to the embryos. The highest water temperature, 27.8°, at which I found an egg mass (in a cistern with little water movement) is well below the base-level tolerance of 31.5°. Temperatures within an egg mass in a shallow stream ranged from 16.1° to 23.6° over several days, but the weather was frequently cloudy in the period of observation. The only "tank" in which I have observed this species is sufficiently deep that high temperatures should not be a problem. The relatively low maximum tolerance of this species, its slow rate of attainment of increased tolerance, and its slow rate of development would seem to place it at a disadvantage in shallow, lowland waters. To what extent this species or others of its species group invades such situations remains to be demonstrated. Seasonal adjustment of the breeding season could be significant. In the Chiricahua Mountains, at least, oviposition takes place over a large part of the year (Zweifel, 1968).

The three species of *Scaphiopus* breed in the summer in desert rain pools, and it is common to find two or even all three species breeding in the same pool at the same time. Therefore, they may be considered together. Brown (1967a) and Zweifel (1968) presented information on temperatures in the breeding habitat in my study area. Eggs are typically deposited at about 19.0-20.0° during the night and by the following afternoon the water temperature may have risen to as high as 35.0°. Brown recorded a maximum of 39.0° (embryos in stage 15). (Occasionally, breeding activity carries over into the daylight hours but at such times the sky is likely to be cloudy and direct insolation of newly laid eggs is a rare event.)

Despite the possibility of being exposed to high temperatures within about 12 hours of oviposition (i.e., by noon following midnight fertilization), *Scaphiopus* embryos stand little danger of injury from this source. At a constant 20.0°, embryos of all three species are well into the gastrulation process in 12 hours (stage 11 or 12) and have attained a two-hour tolerance of at least 39.5°. Of course, their development would be accelerated by rising water temperature as the morning progressed and the effect of rapid heating of the water (as on an unusually warm and sunny morning) would be compensated for by accelerated attainment of maximum adaptation.

The adaptation curve of *Scaphiopus couchii* is particularly striking in relation to environmental temperatures. A *couchii* embryo developing at 20.0° reaches stage 9 in about eight hours, and by late stage 8 already can tolerate 39.7° for two hours. Thus, the embryos can be well prepared for extremely high temperatures before the rays of the sun have had time to affect the temperature of their habitat to any significant degree.

Where *S. couchii* lives in deserts near sea level, as in coastal Sonora, Mexico, breeding undoubtedly takes place in water several degrees warmer than is typical of my study area. But even here it is unlikely that high temperatures pose a serious problem for the embryos, for their more rapid development in warmer water would assure attainment of maximum adaptation well before the heat of the day.

Scaphiopus couchii uses all principal pathways of embryonic adaptation to high temperature. The embryo begins life with the high base-level tolerance of 34.0°. More than 90 percent of the total ontogenetic adaptation to high temperature has been achieved by the onset of gastrulation (stage 10), and the level of two-hour tolerance achieved, 40.3°, is one of the highest. Finally, the developmental rate of *couchii* embryos is faster than that of any other anuran in the same habitat at 24.0° or higher, and below 24.0° is exceeded only by the other two species of *Scaphiopus* (Zweifel, 1968). Clearly, *S. couchii* is extremely well adapted to breed in the summer rain pools of the hottest North American deserts.

Although *Scaphiopus bombifrons* and *S. multiplicatus* do not adapt to high temperatures quite so early in development as does *S. couchii*, they develop more rapidly at low temperatures than does *couchii* (Zweifel, 1968) and achieve a similar net result. They are capable of breeding at lower temperatures than can *couchii* and yet adapt to high temperatures almost as well, so may be regarded as having a broader embryonic adaptation to temperature than does their congener.

The principal feature of interest in *Bufo cognatus* is its high tolerance for elevated temperatures, exceeding that of a true desert species, *S. couchii*. Although occurring in desert regions, *B. cognatus* is more characteristic of the Great Plains grassland. It was studied most thoroughly by Bragg (1937, 1940) in Oklahoma, where he found it usually breeding in shallow, temporary ponds, avoiding deeper, more nearly permanent waters. Although over the whole of its range *B. cognatus* is not so narrowly restricted in choice of breeding sites as Bragg found (Brown and Pierce, 1967), there does appear to be a preference for sites such as Bragg described. Presumably, then, the explanation for the evolution of unusually high temperature tolerance must be sought in the area of breeding microhabitat and breeding habits.

Were eggs of *B. cognatus* and *S. couchii* to be fertilized simultaneously in a rain pool at 20.0° at midnight, by 08:00 the *couchii* embryos would be in stage 9 and would have attained a two-hour tolerance of about 39.7°. In comparison, the *cognatus* embryos would be in stage 8 with a two-hour tolerance perhaps a degree lower. The difference under these conditions is relatively small, and with accelerated development in warming water (and accompanying increase in tolerance level), it is unlikely that heat damage to the *cognatus* embryos would occur. But *cognatus* does not restrict its breeding to relatively cool periods during and soon after rains. *Bufo cognatus* sometimes choruses (and presumably oviposits) independent of rainfall (Brown and Pierce, 1967; personal observ.). At such times temperatures in the breeding ponds may well be considerably higher than those experienced shortly after rain pools are formed by summer thunderstorms. Furthermore, Bragg (1940, p. 21) noted that "egglaying is a long process, often lasting till well into the day following [initiation of] amplexus." Under such conditions, embryos produced in a shallow, temporary pond could be subjected to dangerously high temperatures and, developing at a relatively slow rate (compared with *Scaphiopus*), would be at a distinct disadvantage if they did not possess unusual tolerance for high temperatures.

The rate of embryonic development of *B. cognatus* is not particularly fast in comparison with other *Bufo*. It is, in fact, exceeded by that of the desert species *Bufo punctatus* (*fide* Zweifel, 1968) and the southeastern forms *B. terrestris* and *B. valliceps* (*fide* Volpe, 1953, 1957). Although it may yet be shown that *cognatus* adapts to high temperatures earlier in development than do forms breeding in more temperate habitats, at present it appears that the adaptation principally has taken the form of increased maximum tolerance. It would be particularly interesting to have comparative data for other species of *Bufo*.

Brown (1975) summarized original and published information on temperatures associated with embryos of *Hyla regilla*. The species is a winter and early spring breeder, at least at low elevations, and the majority of records indicate that embryos are not likely to be exposed to temperatures much above 20.0°. (One contrary observation is not documented in detail, unfortunately.) It would be of interest to know more of the embryonic temperature characteristics of this species. Brown (1975) has demonstrated differences in the base-level tolerances of northern and southern lowland populations and has shown also that they exhibit different rates of development. A comparative study of ontogenetic change in tolerance among embryos from vastly different habitats (e.g., desert and high mountain) might prove rewarding.

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