REPRODUCTIVE BIOLOGY OF ANURANS OF THE ARID SOUTHWEST, WITH EMPHASIS ON ADAPTATION OF EMBRYOS TO TEMPERATURE

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BULLETIN
OF THE
AMERICAN MUSEUM OF NATURAL HISTORY
VOLUME 140 : ARTICLE 1 NEW YORK : 1968

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Volume 140, article 1, pages 1–64, text
figures 1–21, plates 1–2, tables 1–10

Issued September 20, 1968

Price: $2.00 a copy

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INTRODUCTION

IT HAS LONG BEEN KNOWN that the embryos of amphibians are injured or even killed by exposure to temperatures that are too high or too low, and that the rate of development of an embryo is to some extent determined by its temperature. Until the appearance of the work by Moore on North American species of *Rana*, however, there was no body of data gathered with an ecological outlook and, of equal importance, none gathered in a standardized fashion that permitted interspecific comparisons. In five papers, Moore (1939, 1942b, 1949a, 1949b, 1952) detailed the embryonic temperature tolerances and developmental rates of six species of *Rana* native to the eastern United States and Canada. He found that for the most part the adaptations of embryos correlated well with the geographic distribution and breeding habits of the frogs. One species, *Rana pipiens*, exhibited considerable geographic variation in tolerances and rates; Moore thought that the exceptional geographic and ecological range of this species was in part at least a reflection of the adaptability of its embryos.

Subsequent work by Moore's students, Volpe (1953, 1954, 1957a, 1957b) and Ruibal (1955, 1962), added valuable information on several species of *Bufo* and contributed to the complex picture of *Rana pipiens*. Herreid and Kinney (1967) studied *Rana sylvatica* in Alaska, a species first investigated by Moore in New York. Studies similar to those of Moore, but far less complete, were made on several American species by Hubbs and Armstrong (1961), Hubbs, Wright, and Cuellar (1963), and Ballinger and McKinney (1966). Among Old World species, *Rana temporaria* of Europe was studied by Douglas (1948), Moore (1951), and Grainger (1959). Douglas (1948) also studied *Rana esculenta* and *Bufo bufo*, and Kobayashi (1962) provided data closely comparable to Moore's on three Japanese species, "*Rana t. temporaria*" (=*R. cruenta*), "*Rana t. ornativentris*" (=*R. ornativentris*), and *Rana japonica*.

All species for which adequate data are available—those studied by Moore, Herreid and Kinney, Volpe and Ruibal, among the American species—are found in relatively mesic regions; only *Rana pipiens* among these invades the deserts of the American Southwest. In view of the paucity of information on species of arid and semiarid regions, I deemed it of interest to study such a fauna, using the techniques and criteria of Moore. The general question for which an answer was sought was: Do the embryos of anuran species living in a hot, semiarid region possess adaptations to temperature that adapt them specifically to this environment? The basic information sought for each species included the upper and lower temperatures limiting for embryonic development and the rate of embryonic development at a variety of constant temperatures.

Field work was concentrated in southeastern Arizona and adjacent New Mexico. The Southwestern Research Station of the American Museum of Natural History provided the essential laboratory facilities. Field and laboratory work took place during the summer months of 1958 and 1960. I studied eight species representing four families: Bufonidae (*Bufo cognatus*, *B. debilis*, *B. punctatus*); Hylidae (*Hyla arenicolor*); Pelobatidae (*Scaphiopus bombifrons*, *S. couchii*, *S. hammondii*); and Ranidae (*Rana pipiens*). The amount of information obtained varied greatly from species to species. Limited by the restrictions imposed by time and equipment, I might have concentrated on fewer species and thereby gathered more information on them, but I preferred to obtain at least some data on as many species as possible.

ACKNOWLEDGMENTS

My field and laboratory work was greatly facilitated by Dr. Mont A. Cazier, then Resident Director of the Southwestern Research Station. Three assistants shared the odd and long hours of laboratory and field work: Mr. William A. Wimsatt (1958), Mr. Charles J. Cole (1960), and Mr. F. Harvey Pough, Jr. (1960). The assistance of Mr. Wimsatt and Mr. Pough was made possible by grants from the Lincoln Ellsworth Memorial Fund of the American Museum of Natural History; Mr. Cole was a participant in the Undergraduate

Research Program sponsored by the National Science Foundation (Grant EO/3/43-1606). National Science Foundation Grant G-5033 provided over-all support for the research program. I thank Dr. John A. Moore and Dr. Charles M. Bogert for their criticism of the manuscript.

METHODS

Experimental Procedure

This consisted of obtaining freshly fertilized eggs, distributing them among several water baths maintained at different, relatively constant temperatures, and noting periodically the stage of development reached.

In some instances frogs found mating in the field were brought to the laboratory where normal oviposition and fertilization took place. Eggs of one species, *Rana pipiens*, collected in the field shortly after having been laid, were then used experimentally. In the majority of experiments, I utilized eggs obtained through induced ovulation and fertilized in a sperm suspension made by macerating the testes of one male. The standard techniques of inducing ovulation by implantation or injection of pituitary glands and of preparing sperm suspensions have been explained by Rugh (1962) and need not be repeated here. For the most part, I used pituitary glands taken from adult females of *Rana pipiens* purchased from a dealer who collected them in the fall or winter. The glands were preserved in absolute alcohol for three to four months before being used. An exception was made in the case of *Scaphiopus*, for which I employed glands (preserved or fresh) of this genus only. Also in some instances with *Scaphiopus*, injection of pituitary glands induced (or hastened) ovulation, but fertilization was natural.

The water baths available in 1958 included two refrigerated, constant-temperature baths and a Warburg apparatus modified to hold trays of eggs. In 1960 four additional water baths became available. These, however, were not refrigerated. Therefore they could be used at constant temperatures only above ambient room temperature, which essentially restricted their use to temperatures above 30° C. The range of variation in the baths rarely exceeded 1° C., and usually the temperature stayed within a range of 0.5° C. In view of the slight fluctuation, only the modal temperature in each experiment is given in the discussions and tables.

Embryos were raised in stream, spring, or rain water in rectangular plastic trays that measured 7 inches in length, 5 inches in width, and 2 1/2 inches in depth. As soon as rotation of the ovum within the envelope indicated that fertilization had taken place, the eggs were placed in the trays and the trays in the baths. As a rule, eggs from one female fertilized by one male were distributed among several trays kept at different temperatures. No set number of eggs was used, but at least 50 were placed in each tray whenever possible.

At intervals the trays were removed from the baths briefly while the eggs were examined microscopically and the stage of development noted; the temperature of the water in the tray was recorded at the same time. Particular note was made of any abnormalities in development, such as failure to incorporate the yolk during gastrulation (exogastrulation) and imperfect or retarded development of the gills.

My experimental procedure differed in one relatively minor respect from that of Moore and other authors cited above, in that I placed the eggs in the water baths as soon as rotation took place, rather than wait until the first cleavage occurred. My own data and those from several published sources indicated that first cleavage occurs after an average of about 2.5 per cent of the total time from fertilization to initiation of gill circulation has elapsed. The importance of avoiding delay in exposing the embryos to the experimental temperatures must be stressed. Resistance to extreme temperatures increases rapidly as cleavage proceeds, so that if embryos are permitted to develop for a time before being subjected to high or low temperatures, the experiment is likely to yield a wider range between lethal temperatures than would be the case with one started at or before first cleavage.

Erratic and evidently abnormal results in some of the experiments conducted in 1958 probably were the result of some polluting factor, possibly a detergent inadvertently

introduced in washing the trays. The data have been carefully screened to exclude any in which such an effect was evident. In general, the effect of the pollutant was more noticeable under stressful situations, that is, at marginally high or low temperatures.

Each experiment involving eggs from one female was assigned a number according to the year and the number of experiments previously done with that species. Thus, for example, experiment 60-1 in table 1 is the first experiment done on *Bufo cognatus* in 1960. This experiment number appears in six places in the table because eggs from this one female fertilized simultaneously in one sperm suspension were distributed among water baths set at six different temperatures.

Staging and Experimental End-Points

The staging system employed was that originated by Pollister and Moore (1937) for the embryonic stages of *Rana sylvatica*. Shumway (1940) adapted the same system to *Rana pipiens* (curiously, without reference to Pollister and Moore), and it was used by Chu and Sze (1957) for *Rana nigromaculata*, by Michniewska-Predygier and Pigón (1957) for *Rana esculenta* and *R. temporaria*, by Ramaswami and Lakshman (1959) for *Rana cyanophlyctis*, by Rossi (1959) for *Bufo bufo*, and by Limbaugh and Volpe (1957) for *Bufo valliceps*. The last authors usefully extended the numbering of states through larval development and metamorphosis, and Gosner (1960) brought together information from several sources in a generalized scheme. Sedra and Michael (1961) provided a table correlating several different staging systems.

I have followed Moore (1949a and other papers), Kobayashi (1962), Ruibal (1955, 1962), and Volpe (1953, 1954, 1955, 1957a, 1957b) in using the time at which 50 per cent of the embryos attain early stage 20, the initiation of gill circulation, as the end-point for experiments concerned with rate of development.

All too frequently in the literature one finds the period of embryonic development discussed only in terms of time to hatching. The time to hatching may be an important datum, but the embryos of every species of anuran do not hatch at the same stage of development, so data for different species based on time to hatching may not be comparable. Embryos of many species of *Bufo*, for example, hatch in stage 16 or 17, whereas some of *Hyla* and *Rana* hatch in stage 20. Also, extrinsic factors can influence the stage at which hatching takes place. Thus, standardization at an easily determined end-point is advantageous in that the results of various experiments may be compared directly. Embryos developing to or past stage 20, and then failing to proceed with normal development, is a rare occurrence. It is reasonable to assume that embryos attaining stage 20 in normal condition will continue to develop normally.

I follow earlier workers in defining the lethal temperatures as those below which (lower) or above which (upper) fewer than 50 per cent of the embryos develop normally. The change from lethal to non-lethal takes place rather abruptly, as the data of Volpe (1957a) for *Bufo valliceps* clearly illustrate.

One potential source of error in determining the end-point at stage 20 must be acknowledged. At experimental temperatures verging on limiting temperatures, the development of the gills and the establishment of circulation in them may be retarded slightly. Thus, the time to gill circulation may actually increase slightly at the highest temperatures tolerated, which does not necessarily mean that the development of the embryo as a whole has been retarded.

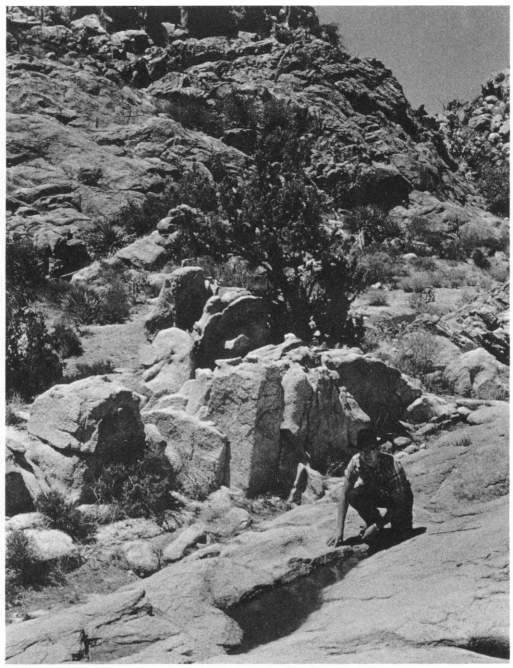
Reasons for Conducting Experiments at Constant Temperatures

Amphibians do not as a rule breed in situations in which the water temperature remains relatively constant throughout embryonic development. There are exceptions, of course, particularly among salamanders that breed in springs or underground waters, but for the most part frogs breed where fluctuations of temperature are usual. In view of this situation, one may question the advisability of determining tolerances and rates of development under constant temperature conditions. The answer is that studies made under both constant and changing conditions are needed (Grainger, 1959). Experiments conducted at constant temperature are readily duplicated and provide basic information on general temperature adaptation that is comparable from species to species. Experiments with

fluctuating or otherwise changing temperatures may be designed to approximate conditions that occur naturally but may be more difficult to standardize. Certainly, it is desirable to establish a base line of data derived at constant temperatures before passing on to studies of varying conditions.

Experiments Conducted at Elevated Temperatures

Most of the experimental work consisted of raising embryos from the start at constant temperatures. In several instances, however, embryos that had undergone some development were placed at a higher temperature, so that we could learn something of the increase in tolerance to high temperatures that takes place as the embryo develops (Atlas, 1935; Moore, 1942b, pp. 380–381, footnote; Schechtman and Olson, 1941). Because temperature baths could rarely be spared for these experiments and because embryonic material was not always available at those times, relatively little was learned. The little that was determined, however, indicates that the subject is worth intensive study. A recent paper by Brown (1967) presents detailed information on the high temperature tolerance of embryos of *Scaphiopus hammondii* as influenced by the stage of development of the embryo and points the way for future studies of a comparative nature.



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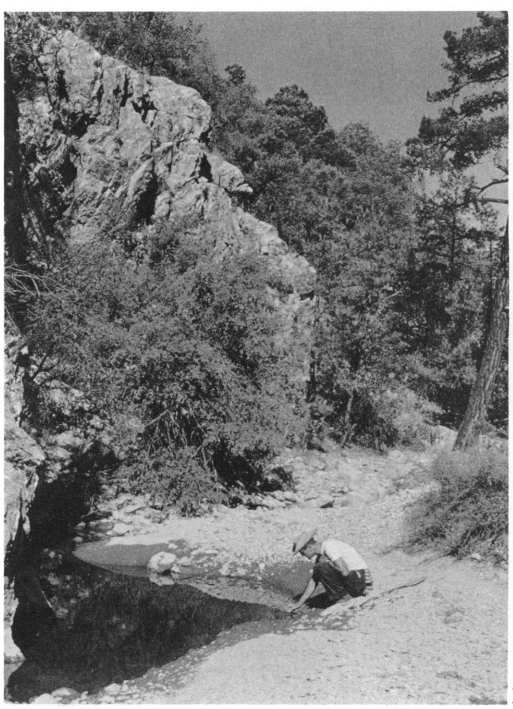

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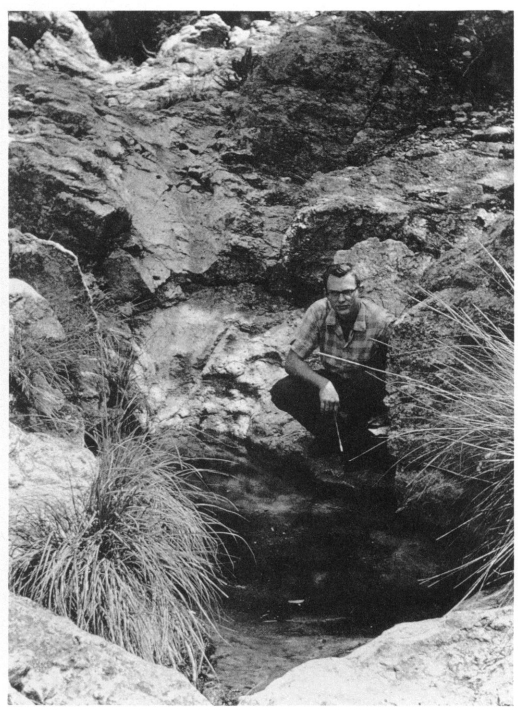
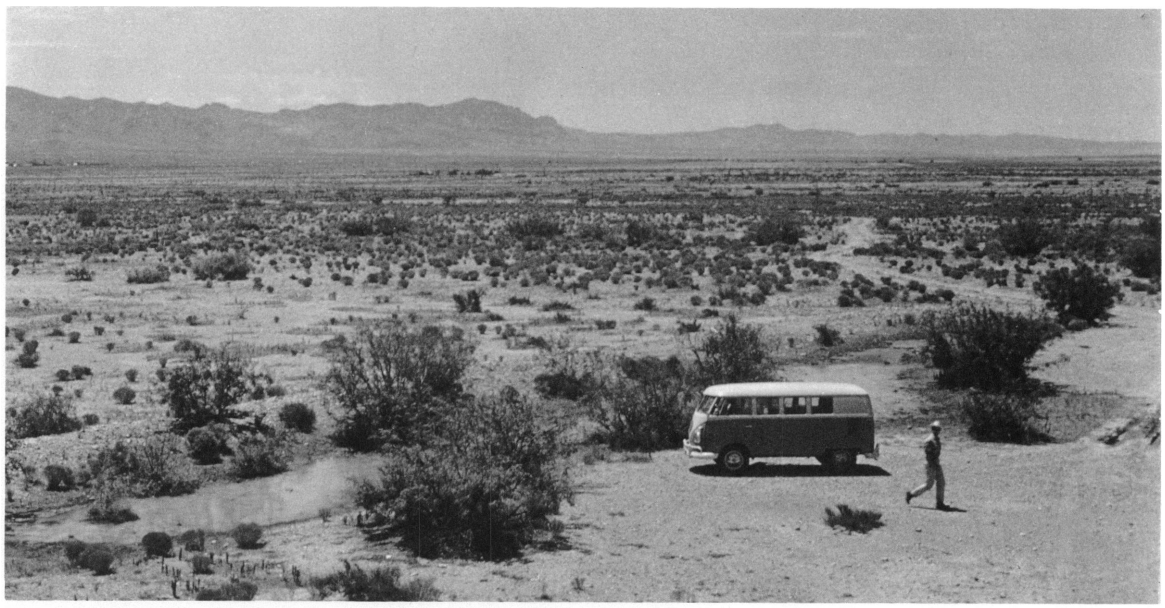


3

1. Breeding site of *Bufo punctatus*, shallow pool in granite outcrop at Granite Gap, Peloncillo Mountains, 4400 feet, Hidalgo County, New Mexico, July 12, 1960. 2. Breeding site of *Rana pipiens* and *Hyla arenicolor*, stream in Clanton Canyon, Peloncillo Mountains, 5600 feet, Hidalgo County, New Mexico, July 15, 1960; *Bufo punctatus* and *Scaphiopus hammondii* also present. 3. Breeding site of *Hyla arenicolor*, pothole in bed of intermittent tributary to Cave Creek, 5400 feet, Chiricahua Mountains, Cochise County, Arizona, July 18, 1960



1

2

Rain pool in San Simon Valley, 6 miles east-southeast of Portal, 4140 feet, Cochise County, Arizona. Breeding site of *Scaphiopus bombifrons*, *S. couchii*, and *S. hammondii*. *Bufo debilis* and *B. cognatus* also occur here and presumably breed in an adjacent cattle tank. 1. Photographed July 11, 1960; high-water mark shows extent of pool when filled by storm on evening of July 8. 2. Photographed July 13, 1960; all surface water was gone by July 16, and a brood of *Scaphiopus* produced on July 8 was exterminated

DISTRIBUTION AND HABITATS

STUDY AREA AND BREEDING HABITATS

THE STUDY AREA (the region in which most of the experimental animals were captured) includes the Chiricahua Mountains, San Simon Valley, the Peloncillo Mountains, and Animas Valley. The Chiricahua Mountains are in Cochise County of southeastern Arizona. The southern end of San Simon Valley flanks the mountains on the east and is bisected by the Arizona-New Mexico boundary. Animas Valley in Hidalgo County, New Mexico, is the next valley to the east and is separated from San Simon Valley by the low Peloncillo Mountains.

The Chiricahua Mountains are about 15 miles west of the Arizona-New Mexico boundary and 35 miles north of the boundary between the United States and Mexico. San Simon Valley on the east and Sulphur Springs Valley on the west of the mountains (no collecting was done there) give way rather abruptly to mountain slopes which begin at a basal elevation of about 5000 to 6000 feet. The mountains reach a maximum elevation of 9795 feet. The main mountain mass measures about 20 miles from east to west and 35 miles from north to south. Although the eastern and western edges of the mountains are sharply defined where they meet the valleys, the Chiricahuas are separated only by low passes from lower and less massive mountains to the northwest and south.

The Chiricahua Mountains present varied vegetation, ranging from fir forests at high elevations through pine forests and pine-oak woodland to the oak woodland of the lower slopes. The amphibian life does not reflect this diversity of vegetation. I found only two species of frogs breeding in the mountains: *Rana pipiens* and *Hyla arenicolor*.

Because of the rugged, well-drained topography and semiarid climate, the mountains are relatively poor in aquatic habitats that would provide breeding sites for frogs. Natural standing-water habitats occur only where erosion has produced potholes in the beds of intermittent streams or where pools remain following the disappearance of surface flow in a normally permanent stream. Man-made dams, however, impound small lakes in several places in the mountains.

The larger streams normally experience a period of flow in the spring, the water being derived from melting snow at high elevations. The flow generally diminishes as summer approaches, and by early summer, even in the larger canyons, water can be found only in isolated pools or in spring-fed segments of the watercourse. Streams, whether permanent or intermittent, are subject to irregular and violent fluctuations in flow during the summer months when thunderstorms send flash floods churning down the canyons. Because the storms are highly localized, one stream may flood while the stream in the next canyon may be unaffected. Similarly, one stream may flood several times in a summer and another not at all. I refer the reader to John (1964) for an excellent description of the stream habitat in the Chiricahua Mountains.

The volume of water in the streams and the period in which water is present not only fluctuate throughout the year and from year to year, but also appear to be influenced by long-term climatic trends. For example, in the early 1900's Cave Creek (the largest stream on the east side of the Chiricahua Mountains) maintained flow throughout the year past the town of Portal at the foot of the mountains, but permanent water is now 5 miles upstream from Portal (John, 1964, p. 117). Such large-scale changes in the availability of water must greatly influence the population density of frogs and may influence species composition of the population as well.

Most of the species used in the experiments occur in the valley habitats, and the majority of experimental animals were captured in the southern part of the San Simon Valley between the towns of Portal, Arizona, and Rodeo, New Mexico. The valley in this region is 8 to 10 miles wide. The elevation at Portal at the edge of the Chiricahua Mountains is 4773 feet, and the base of the Peloncillo Mountains across the valley lies at about 4400 feet; the minimum elevation of the valley floor between these points is approximately 4100 feet. The vegetation of the valley

in this region is basically Desert-Grassland (as characterized by Lowe, 1964a, pp. 40–43), although it is not uniform. Mesquite (*Prosopis juliflora*) thickets occupy some areas, notably around Portal and the mouth of Cave Creek Canyon, and typical Chihuahuan Desert vegetation dominated by creosote bush (*Larrea divaricata*) occupies coarser soils on both eastern and western edges of the valley.

Natural breeding sites for frogs occur only where depressions fill with water during summer storms. Such natural sites are not abundant and are far outweighed in importance by artificial pools, so-called cattle tanks. These pools are merely scooped out of the fine soil, often with the excavated soil deposited to form a dam across an intermittent watercourse. The more important watering holes are provided with wells and wind-driven pumps, but lesser ones rely on rainfall and thus may dry up. Although artificial in that they owe their existence to the activity of man, these pools resemble natural rain pools in that they are mud-bottomed and turbid and are used seemingly without prejudice by the anurans that normally breed in rain pools. I think it likely that because of the existence of relatively large numbers of these pools (11 large enough to map appear in 25 square miles northeast of Portal on the 1:62,500 topographic sheet, for example), the anuran population is higher than it would be under primitive conditions. Not only is the number of potential breeding sites multiplied, but their reliability in retaining water for a sufficient length of time to permit metamorphosis is enhanced.

Breeding sites similar to those of San Simon Valley are found also in Lower Animas Valley which is similar in elevation (4200–4300 feet) and in variety of vegetation. No experimental animals were collected in the Peloncillo Mountains which are very poorly watered.

LOCAL DISTRIBUTION OF FROGS

This brief discussion and the foregoing account of habitat conditions provide the ecological setting for considerations of the significance of the limiting temperatures and developmental rates. Unless otherwise specified, the discussion deals only with distribution and habitat within the study area.

BUFO COGNATUS

This species is common throughout the San Simon Valley and also occurs in Animas Valley. It was not found in upland habitats. The frequent association of *B. cognatus* with cattle tanks, irrigation ditches, and flooded fields (Lowe, 1964b, p. 156; Brown and Pierce, 1967) suggests that this species has benefited from man's agricultural activities.

BUFO DEBILIS

The distribution of this species is similar to that of *B. cognatus* in that it occurs broadly throughout the valleys and up to the edge of the mountains, as at Portal, where Cave Creek leaves the Chiricahua Mountains. It does not penetrate the mountains.

BUFO PUNCTATUS

Records for this species are spotty and do not give a clear picture of its distribution. It was abundant around an artificial pond at Portal, where there was a permanent water supply, and could occasionally be found at a cattle tank 1 mile to the west. Both localities are at the very edge of the mountains. It is present in Granite Gap in the Peloncillo Mountains, where water is found only intermittently in rain-filled potholes (pl. 1, fig. 1) and near the summit of the Peloncillo Mountains at 5600 feet in elevation in Clanton Canyon in pine-oak woodland (pl. 1, fig. 2). Judged from the presence of *Rana pipiens* here, water is probably present all year. Occasionally an individual of *punctatus* appears in lowland habitats, as attested by a specimen captured 1 mile north of Apache in the San Simon Valley, a locality more than 1 mile from the foot of the mountains.

My assistants and I spent many hours collecting in Cave Creek Canyon in habitats at least superficially similar to those where *B. punctatus* occurs elsewhere (as in Clanton Canyon) without finding these toads. Perhaps the terrestrial habitat there is inhospitable in some respect, for the potential breeding sites appear to be suitable. It may be that in decades past, when Cave Creek flowed to and past the mouth of the canyon, *Bufo punctatus* lived and bred in the lower part of the canyon and the population living now around Portal is a relic of that moister period.

Hyla arenicolor

Hyla arenicolor breeds in both permanent and intermittent streams (Zweifel, 1961); that is, in a stream that retains at least disconnected pools throughout the year (pl. 1, fig. 2) and in a stream in which even the deeper potholes dry up during persistently dry weather (pl. 1, fig. 3). Although confined to upland habitats, this species ranges widely therein and at least in the summer rainy season may be found away from permanent sources of water. For example, one was taken on the rock slide near the summit of Barfoot Peak, approximately 8400 feet in elevation.

Rana pipiens

In the study area *Rana pipiens* is found only at permanent sources of water, and is confined to the mountains. Man-made, still-water habitats, such as swimming pools and small lakes impounded behind dams, serve as breeding sites. This species also breeds in permanent streams where it is frequently abundant, although I did not happen to find eggs in such a site in the Chiricahuas. In well-watered sites in the valleys such as at San Bernardino Ranch on the Mexican border, *pipiens* is abundant. I did not find individuals of *pipiens* in cattle tanks in San Simon Valley, even though the water supply was permanent. Perhaps the scarcity of pond-side vegetation is a contributing factor, for the more thoroughly aquatic (and introduced) *Rana catesbeiana* was present in at least one tank.

Scaphiopus bombifrons, S. couchii, and S. hammondii

These species are widely and sympatrically distributed throughout the valleys, where they breed in cattle tanks and rain pools. Apparently only *Scaphiopus hammondii* ranges into low montane habitats. I found one near the summit of Clanton Canyon in the Peloncillo Mountains (pl. 1, fig. 2; elevation 5600 feet), but no individuals of this species appeared in similar sites in the Chiricahua Mountains. It is difficult to imagine where these frogs would find suitable breeding sites in the Chiricahuas (or in the Peloncillos, for that matter).

Other Species

When the field work was completed in 1960, it was thought that data had been gathered on all species native to the study area. Surprisingly, in 1961 *Bufo alvarius* appeared and was found in moderate abundance in areas that had been intensively searched in earlier years (see Cole, 1962, for details).

On the basis of its over-all geographic distribution, *Bufo woodhousii* might be expected in the study area. In arid regions it is commonly associated with watercourses (Lowe, 1964b, p. 156), and no adequate habitat appears to be present in my study area. At San Bernardino Ranch in extreme southern Cochise County I found numerous newly metamorphosed toads probably of this species, although no adults were obtained for confirmation of the determination.

LIMITING TEMPERATURES AND DEVELOPMENTAL RATES

BUFO COGNATUS

THE EXPERIMENTAL ANIMALS were collected in the San Simon Valley and northern Animas Valley. Males in breeding condition could be found in ditches in irrigated areas and calling among other anurans at cattle-watering tanks. We encountered no breeding females in these situations, however, and had to use individuals found on roads on rainy nights. Those in condition to respond ovulated within about seven hours after injection of three or four preserved pituitary glands of female *Rana pipiens*.

LIMITING TEMPERATURES

LOWER LIMITING TEMPERATURE

There was no normal development in two experiments conducted at approximately 11° C. In one of these only the initial cleavage took place; in the other, development continued to stage 7 or stage 8 before all the embryos died, but cleavage was highly irregular and eccentric. One experiment at 13.6° C. produced a similar result; no embryo developed beyond stage 7, and cleavage was abnormal.

Five experiments conducted at 15.6° C. produced variable results, indicating that this temperature is close to the threshold for normal development. Almost all embryos of one experiment survived to stage 20, although initiation of gill circulation may have been delayed. Embryos in another experiment showed some abnormality in the form of slight exogastrulation, and eventually suffered 50 per cent mortality. In two experiments very few embryos survived past gastrulation, and in one other there was highly irregular cleavage, with no development past stage 7. Development was normal at the next higher temperature, 20.1° C. In view of the situation observed at 15.6° C., I estimate the lower level for normal development to be approximately 16° C.

UPPER LIMITING TEMPERATURE

In one experiment at 35.0° C. the majority of the embryos died in cleavage stages, and none survived beyond stage 15. Approximately 50 per cent of the embryos in an experiment at 33.6° C. lived to stage 20. Eggs that experienced a temperature range of from 33.4° to 33.7° C. from stage 8 to stage 12 produced normal larvae but showed some slight exogastrulation in stage 12, whereas others of the same clutch kept at from 32.4° to 32.6° C. showed no sign of abnormal development. The development of other embryos at 33.1° C. and lower temperatures was entirely normal. The upper limiting temperature is estimated to be 33.5° C.

RATE OF DEVELOPMENT

The average elapsed time to stage 20 in three experiments at 15.6° C. was 241 hours (range, 234–247 hours). This temperature is slightly below the estimated minimum for normal development, 16° C., a temperature at which development should take about 220 hours. At the upper limit of temperature tolerance at about 33.0° to 33.5° C., development takes approximately 24 hours (fig. 1).

Bufo cognatus is typical of the genus in that its embryos hatch at a relatively early stage of development. Embryos in three experiments hatched in stage 16. In another experiment two groups of embryos kept at different temperatures commenced hatching at slightly different stages, late stage 16 and stage 17, and hatching was delayed until early stage 17 in another experiment. The stage at which hatching takes place does not appear to correlate with temperature, for embryos kept at the highest and lowest temperatures, 32.0° and 15.6° C., hatched in stage 16. The time to hatching is from 50 per cent to 67 per cent of the time to stage 20, but because of individual variation (even within a single clutch of eggs) precise time limits cannot be given. The "hatching" curve in figure 1 is merely an approximation.

COMPARISON WITH PREVIOUSLY PUBLISHED RESULTS

Some information on *Bufo cognatus* was presented by Ballinger and McKinney (1966). These authors stated that the "minimum

TABLE 1

RESULTS OF EXPERIMENTS ON *Bufo cognatus*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
58-1	11.0	—	—	No development beyond first cleavage
58-3	11.2	—	—	No development beyond stage 8
60-1	13.6	—	—	All died in early cleavage stages
58-1	15.6	—	—	Normal to gastrulation, but none survived beyond stage 14
58-3	15.6	234	4.3	Normal to gastrulation, but most died in that stage; 37 of 282 (13%) attained stage 20
58-4	15.6	—	—	No development beyond irregular early cleavages
58-5	15.6	241	4.1	High survival through stage 12 with slight exogastrulation, but only 83 of 167 (50%) reached stage 20
58-6	15.6	247	4.1	Normal development
60-1	20.1	97.5	10.2	Normal development
58-6	20.3	86	11.6	Normal development
58-1	24.5	51	19.6	Normal development
58-3	24.5	48	20.8	Normal development
58-4	24.5	52	19.7	Normal development
58-5	24.5	50	20.0	Normal development
60-1	25.8	43	23.2	Normal development
58-6	29.4	27.5	36.4	Normal development
58-4	31.5	30	33.3	Normal development except for slight exogastrulation that did not persist
60-1	32.0	25.5	39.2	Normal development
60-1	33.1	24	41.7	Normal development
60-1	33.6	24	41.7	About 50 per cent survival to stage 20
58-5	35.0	—	—	Most embryos died before reaching stage 9, one lived to stage 15

lethal temperature of *B. cognatus* is near 14.0°, while the maximum lethal temperature is above 34.5° but below 39.1°" (1966, p. 24).

Because of the manner in which Ballinger and McKinney presented their data (to compensate for small sample size, temperature data were pooled at intervals of 4° C.), their experimental results cannot be directly compared with mine. In table 2 of Ballinger and McKinney, 50 per cent of 20 embryos in the temperature range from 14.1° to 18.0° C. and only 30 per cent of 10 embroys raised at 34.1° 38.0° C. hatched. There is no information on how the embryos were distributed through the temperature ranges (how many were raised at what temperatures), so it is not clear how they determined the limits cited. If only 30 per cent of the embryos raised above 34.1° C. hatched, it is difficult to see how the maximum lethal temperature can be "above 34.5°" if the 50 per cent viability criterion is adopted, as the authors profess to do (p. 22). The reasons for setting the lower limit at "near 14°" are similarly obscure, if only 50 per cent of the embryos through the whole range of from 14.1° to 18.0° C. survived to hatching stage. The statement (p. 24) "Normal development occurred in 6 to 13 days at temperatures from 15.9° to 20.8° with only a single individual failing to hatch at these temperatures" is in better agreement with my determination of the lethal minimum at approximately 16° C.

Ballinger and McKinney (1966, p. 24)

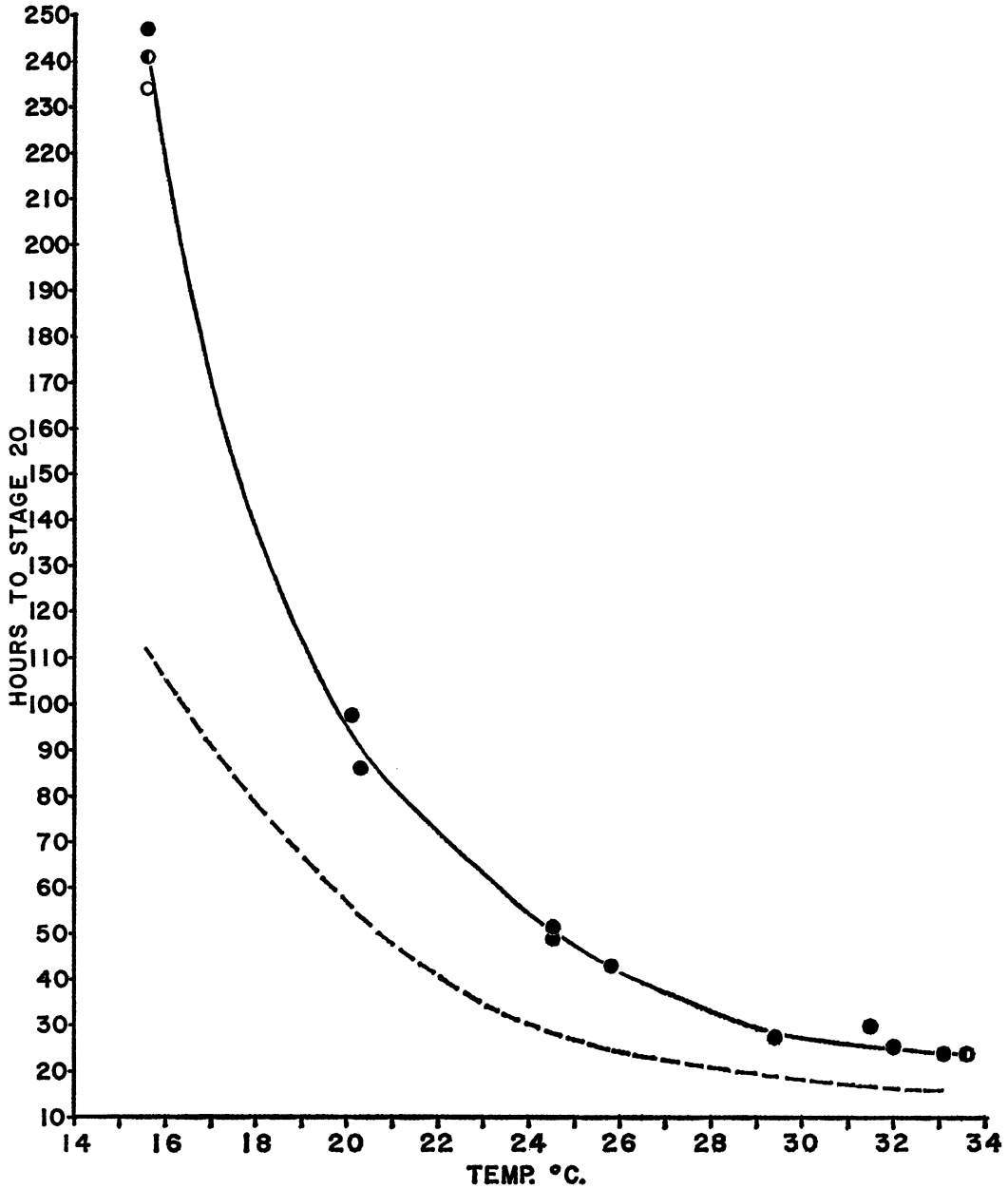


FIG. 1. Time required by embryos of *Bufo cognatus* to hatch (broken line) and to reach stage 20 (solid line) when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

Symbols: Solid symbols, normal development; open circle, less than 50 per cent survival; half-filled circles, approximately 50 per cent survival.

stated, "At temperatures of 21.6° to 25.7° hatching occurred in 4 to 5 days and three days at temperatures of 29.4° to 34.5°." Their table 2, however, indicates that hatching took place in "2.0±0.00" days at 30.1° to 34.0° C. No matter which figures are correct, their results are at extreme variance from mine. In two of my experiments at

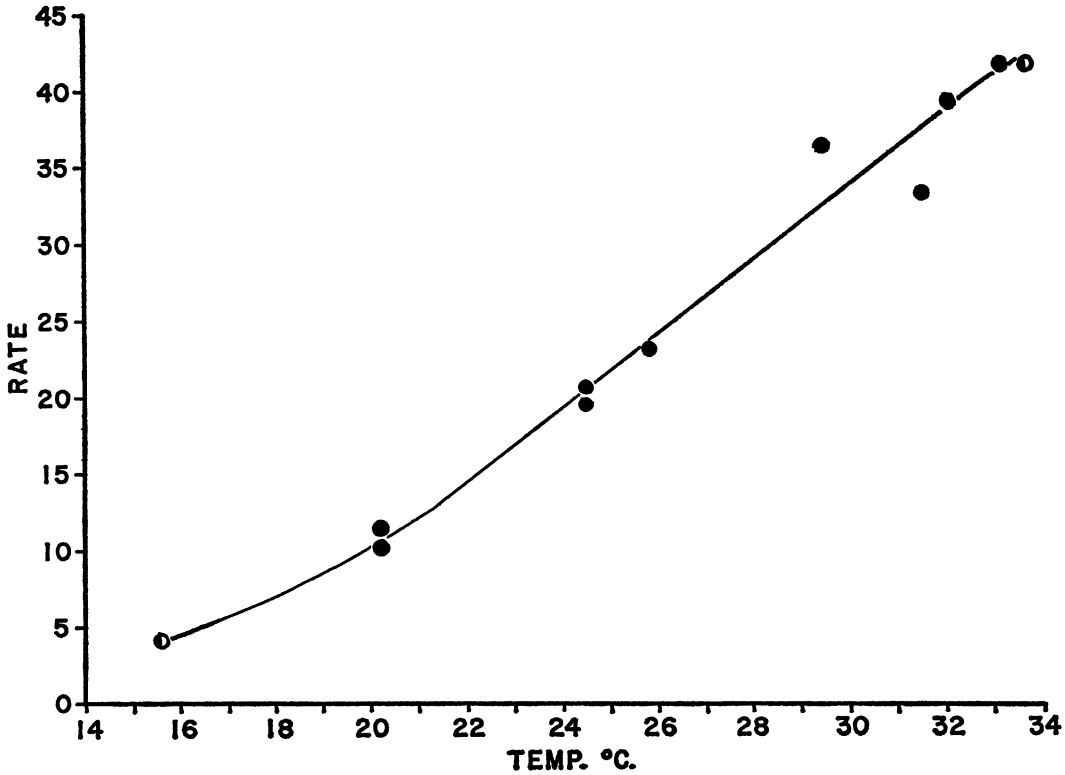


FIG. 2. Rate of development of embryos of *Bufo cognatus* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 ($1/T \times 1000$).
Symbols: Solid symbols, normal development; half-filled circles, approximately 50 per cent survival.

31.5° C. and 32.0° C., hatching commenced at about 16 to 17 hours, and the discrepancy is of similar proportion at other temperatures. In fact, the times cited by Ballinger and McKinney are far in excess of the time required to stage 20 at the temperatures cited.

Confirmation of the time to hatching that I observed is found in a statement by Bragg (1940, p. 21): "At ordinary temperatures, hatching occurs in something over fifty hours (fifty-three in some laboratory cultures. . .)." Presumably by "ordinary temperatures" Bragg referred to room temperatures in the vicinity of 20° C. The "hatching" curve for *cognatus* (fig. 1) indicates an elapsed time of 56 hours at 20° C. Hatching in this species of *Bufo* is a rather inconspicuous process, since the larvae at hatching are virtually immobile. Perhaps Ballinger and McKinney recorded "hatching" only when the larvae obviously were moving or even swimming, hence several hours after the actual hatching. Also, some of the disagreement between our results may be due to differences in precision of recording time. That is, Ballinger and McKinney may merely have noted the day of hatching, whereas I attempted to determine the time to the nearest hour.

BUFO DEBILIS

Most experimental animals came from the vicinity of Rodeo and Portal in the San Simon Valley, but we collected a few near Douglas, Cochise County, Arizona, some 40 miles southwest of the Rodeo-Portal region. In most instances injection or implantation of two preserved pituitary glands of female *Rana pipiens* was sufficient to induce ovula-

tion within about four hours. On one occasion natural ovulation and fertilization took place when a pair in amplexus were brought into the laboratory, but artificial fertilization by sperm suspension was used in all other experiments.

Bufo debilis was by far the most difficult species with which to work. It sometimes proved difficult to strip the eggs, and those obtained were crushed and broken. In such instances a small number of fertilizable eggs were taken by dissecting them from the terminal part of the oviduct.

The eggs of *debilis* have relatively little dark pigment compared with those of most species of *Bufo*, and the cleavage furrows are difficult to see. In fact, it was sometimes difficult or even impossible to determine the stage of development except in the early and late stages.

The toads of my study area belong to the subspecies *Bufo debilis insidior* (Bogert, 1962). For the sake of brevity, I use the specific name only.

LIMITING TEMPERATURES

Lower Limiting Temperature

Data are insufficient for the establishment of the lower limiting temperature except they indicate that it lies in the range of from 16° to 18° C., probably nearer the lower figure. In one experiment at 15.6° C. the embryos seemed to develop normally, but, although hatching, they never developed gill circulation. At 18.2° C. development proceeded normally until most of the embryos died at about stage 16, probably from effects of pollution rather than from damage by low temperature. Normal development took place at 18.8° C.

Upper Limiting Temperature

Fewer than 50 per cent of the embryos of an experiment at 33.0° C. survived, but all the experiments involving eggs from this particular female showed high mortality. Another, more viable group of embryos gave normal development at 33.2° C. and 33.6° C. Embryos from the first group showed high mortality at 33.9 C., but a third group at 34.1° C. had less mortality, though still above 50 per cent. In view of the good survival rate at 33.6° C. and partial survival at 34.1° C., the upper limiting temperature may be approximately 33.8° C.

RATE OF DEVELOPMENT

The minimum temperature at which embryos were brought to stage 20 was 18.2° C.; the elapsed time was 162 hours. To judge from the trend of the time-temperature curve (fig. 3), perhaps 220 hours or more would elapse to stage 20 at 17° C. Embryos kept for 250 hours at 15.6° C. never developed gill circulation. The highest rate of development was reached by embryos raised at 33.6° C.; these attained stage 20 in 23 hours.

Bufo debilis and its relatives *B. kelloggi* and *B. retiformis* differ from other species of *Bufo* in that the embryos hatch at a later stage of development, although still not so late as is characteristic of many anurans with "typical" aquatic embryonic development. Hatching may take place as early as stage 18, but in most instances I observed that hatching occurred in stage 19. Occasional individuals remained in the capsules well into stage 20. The "hatching" curve in figure 3 gives a rough approximation of the time at which hatching may take place, ranging from about 140 hours at 18.2° C. to 22 hours at 33.6° C.

BUFO PUNCTATUS

All male toads and all but one female used in the experiments came from an artificial concrete pond at Portal. The exceptional female was captured about 30 miles north of Portal. Inducing these toads to ovulate was no problem. All those from Portal were in amplexus when captured; two were permitted to ovulate naturally, and the others responded within seven to 10 hours to the implantation or injection of one or two preserved pituitary glands of *Rana pipiens*.

LIMITING TEMPERATURES

Lower Limiting Temperature

There was no development in experiments conducted at 12.8° C. and at 13.6° C. The next higher temperature at which experiments were conducted, 17.1° C., gave no

TABLE 2

RESULTS OF EXPERIMENTS ON *Bufo debilis*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
58-3	15.6	—	—	High survival, but gill circulation never developed (followed to 250 hours)
58-5	18.2	162	6.7	Development normal through stage 16, but almost all died before stage 20; rate based on four survivors
60-2	18.8	130	7.7	18 of 39 embryos (46%) died in pregastrula stages; remainder reached stage 20
60-3	20.1	92	10.9	Normal development
58-3	21.0	88	11.4	Normal development in one tray, 36 per cent mortality in another
58-5	21.0	93	10.7	Development normal through stage 18, but only 10 of 114 embryos reached stage 20
60-1	21.0	103	9.7	Low survival (12 of 20 embryos) not attributable to temperature
58-1	24.4	50	20.0	Low survival (17 of 54 embryos) not attributable to temperature
60-3	26.0	39	25.6	Normal development
58-3	29.4	31.5	31.7	Normal development
60-2	30.5	27	37.0	41 of 55 embryos (75%) survived to stage 20
60-2	32.0	24	41.7	Normal development
60-3	32.0	25	40.0	Normal development
60-1	33.0	24	41.7	22 of 34 embryos (64%) died in early cleavage
60-3	33.2	23.5	42.5	Normal development
60-3	33.6	23	43.5	Normal development
60-1	33.9	—	—	No survival, 35 of 40 embryos died in early cleavage
58-1	34.1	25	40.0	Fewer than 50 per cent survived to stage 20
58-5	35.3	—	—	Approximately 50 of 100 embryos died in stage 9; 11 survived beyond stage 12; 8 reached stage 20
60-1	35.6	—	—	No survival beyond earliest stages

unusual mortality, but one group of eggs showed slight exogastrulation for a time. From this indication of a deleterious effect of low temperature, I infer that the lower limiting temperature is not much below 17° C., perhaps about 16° C.

UPPER LIMITING TEMPERATURE

Two experiments at 32.2° C. produced no evidence of heat damage, but there was 22.5 per cent mortality in one of two experiments at 32.6° C. Experiments at 32.8° C. to 33.8° C. gave variable results: in one experiment each at 32.8° C., 33.7° C., and 33.8° C. there was high mortality, but in one experiment at 33.0° C. more than 50 per cent of the embryos survived, and in another at 33.8° C. there was approximately 50 per cent survival. The upper limiting temperature may be set at 33.0° C.

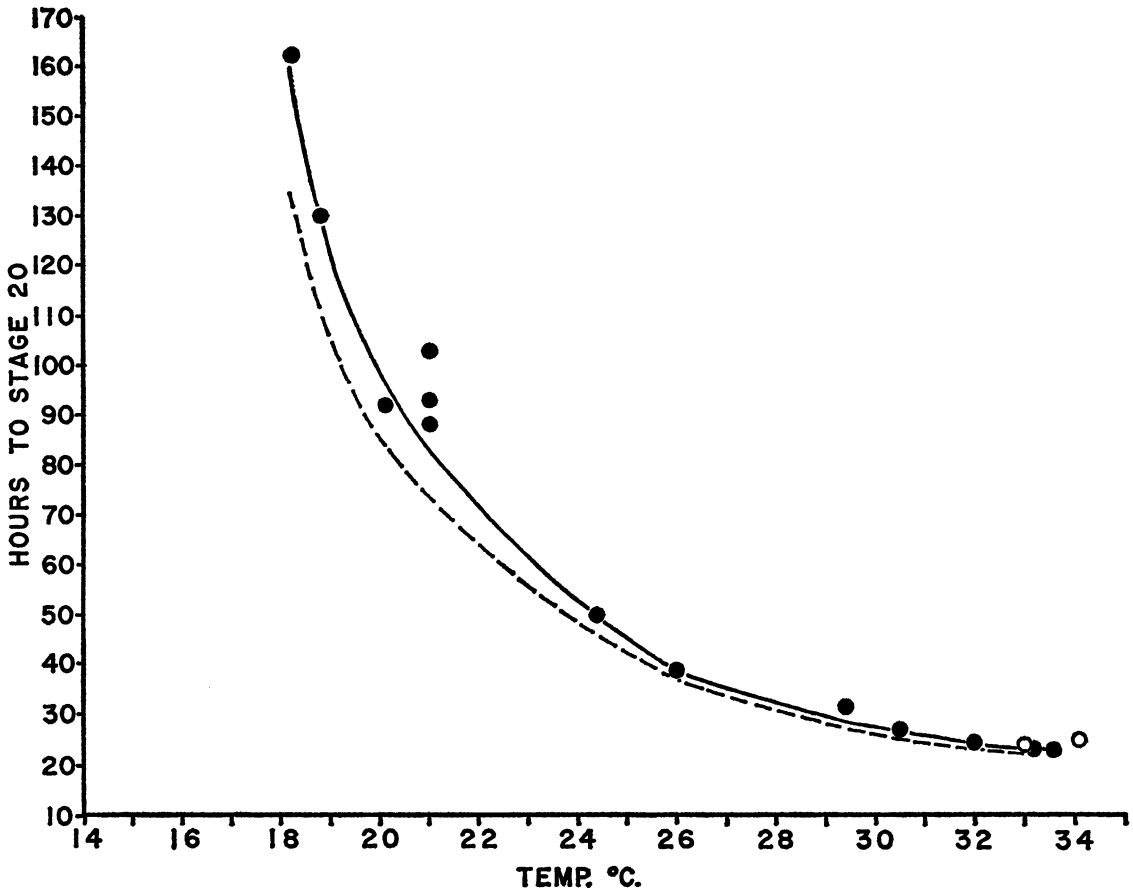


FIG. 3. Time required by embryos of *Bufo debilis* to hatch (broken line) and to reach stage 20 (solid line) when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

Symbols: Solid symbols, normal development; open circles, less than 50 per cent survival.

EFFECT OF EXPOSURE TO HIGH TEMPERATURE IN CLEAVAGE STAGES

In one experiment, embryos raised to stage 8 at room temperature were transferred to 34.8° C., about 1° to 1.8° C. higher than could be tolerated in the early stages. These embryos developed beyond stage 20 with no evident defects.

Another group of embryos raised at room temperature to early stage 7 was placed in water of 36.4° C. This temperature was maintained for 16 hours when an eight-hour power failure allowed the temperature of the water bath to drop to 29.8° C. The original temperature was restored shortly thereafter, but the temperature again fell to 34.1° C. by the end of the experiment at 31 hours.

The embryos in this experiment developed normally and attained stage 20 at 31 hours after having first been placed at the elevated temperature. Inasmuch as the power failure did not take place until 16 hours after the embryos in stage 7E were placed at the high temperature, it is certain that the critical period of gastrulation was passed successfully at a temperature of about 36.4° C. The embryos were in early stage 17 shortly after the restoration of electric power and passed the remainder of their development in the temperature range of 34.0° to 36.4° C.

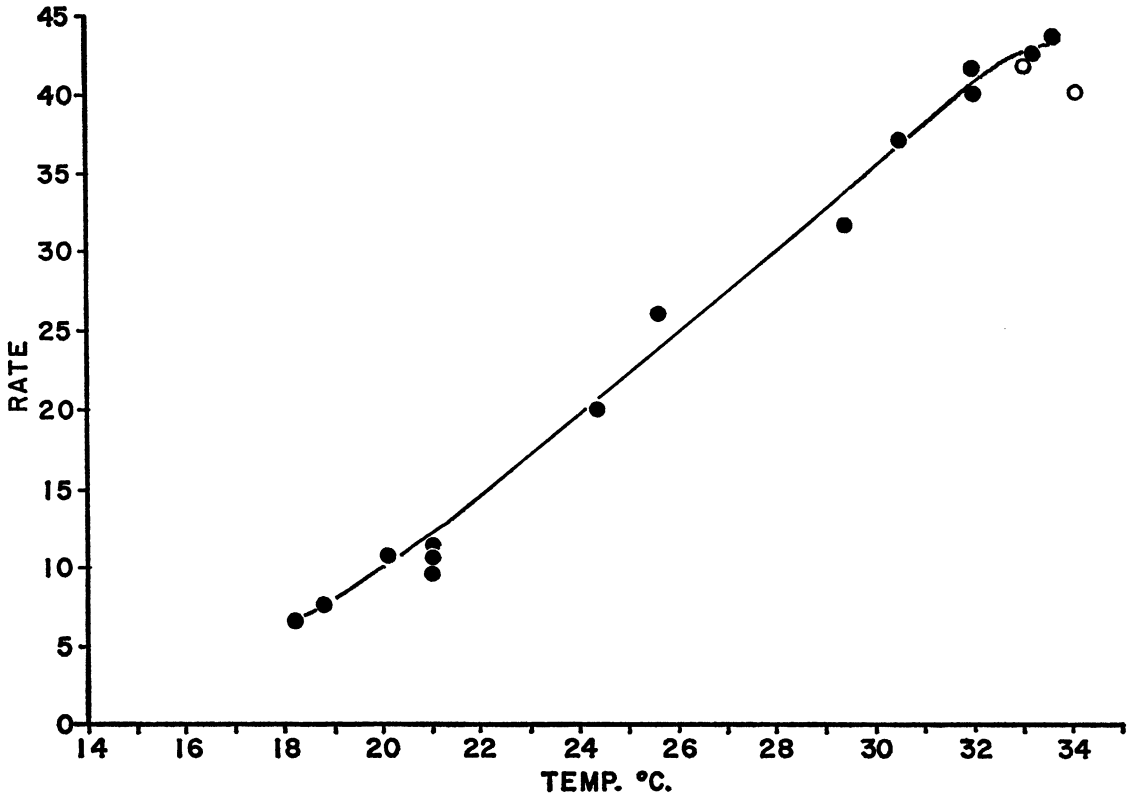


FIG. 4. Rate of development of embryos of *Bufo debilis* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 (1/T×1000).
Symbols: Solid symbols, normal development; open circles, less than 50 per cent survival.

Another group of embryos exposed to the same thermal fluctuation but starting at a slightly later stage of development, middle stage 8, also developed normally to stage 20 in a similar length of time.

RATE OF DEVELOPMENT

In two experiments at the lowest temperature at which normal development took place, 17.1° C., the elapsed time to stage 20 was 155 and 175 hours. Evidently this temperature is near the lower limit for normal development. Judged from the time-temperature curve (fig. 5), reducing the temperature as little as 0.5° C. would extend the development time to about 200 to 210 hours.

Most rapid development took place at 33.8° C., with 21 to 21.5 hours elapsing in two experiments. This temperature, however, appears to be slightly higher than the maximum for normal development. The fastest rate observed within the normal range was 22.5 hours at 32.2° C.

It is curious that embryos exposed to high but fluctuating temperatures (see foregoing section) took much longer to develop than those raised at moderately high but constant temperatures. They took longer, in fact, than would be expected if they were kept at a constant temperature equal to the lowest point reached in the fluctuations.

The embryos of *Bufo punctatus* hatch at a relatively early stage of development, but possibly later, on the average, than those of *B. cognatus*. In five of six instances noted *punctatus* hatched in stage 17; hatching in the other case took place in stage 16.

TABLE 3

RESULTS OF EXPERIMENTS ON *Bufo punctatus*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
60-5	12.8	—	—	No development
60-6	13.6	—	—	No development
60-1	17.1	175	5.7	Normal development
60-2	17.1	155	6.4	Slight exogastrulation, but almost 100 per cent normal survival
60-5	19.5	90	11.1	Normal development
60-6	20.0	83	12.0	Normal development
60-1	21.1	76	13.1	Normal development
60-2	21.1	76	13.1	Normal development
60-3	23.0	55	18.2	Normal development
60-4	23.0	55	18.2	Normal development
60-6	26.2	40.5	24.7	Normal development
60-4	28.3	30	33.3	Normal development
60-3	28.4	30	33.3	Normal development
60-3	32.2	22.5	44.4	Normal development
60-4	32.2	23	43.5	Normal development; 90 of 108 embryos (83%) normal at stage 20
60-1	32.6	25	40.0	Normal development
60-2	32.6	24	41.7	Normal development; 124 of 160 embryos (78%) normal at stage 20
60-5	32.8	—	—	59 of 61 (97%) died in cleavage or early in gastrulation
60-1	33.0	25.5	39.2	60 of 82 (73%) survived to stage 20
60-2	33.7	24	41.7	39 of 171 (23%) survived to stage 20
60-3	33.8	21.5	46.5	Approximately 50 per cent survived to stage 20
60-4	33.8	21	47.6	44 of 123 (36%) normal at stage 20; 16 deformed survivors and 63 dead in early stages
60-5	34.0	—	—	100 per cent mortality, most occurred in cleavage stages
60-1	34.3	26.5	37.7	50 of 250 (20%) survived to stage 20
60-2	35.0	—	—	Most embryos died prior to stage 12; 3 of 170 (1.8%) reached stage 20
60-3	35.6	—	—	Most embryos died prior to stage 12; 8 of 127 (6.3%) reached stage 20
60-5	36.4	—	—	All died in cleavage stages

COMPARISON WITH PREVIOUSLY PUBLISHED RESULTS

Ballinger and McKinney (1966) studied embryonic temperature tolerances and developmental rates of *Bufo punctatus* from Maricopa County, Arizona, and Wimberley County, Texas. They estimated that the minimum lethal temperature for the Arizona embryos was below 18° C., whereas that for the embryos from Texas was about 21° C. The temperature stated for the Arizona embryos is in agreement with my findings. Intraspecific geographic variation in embryonic temperature tolerance is not unknown in *Bufo*, but Ballinger and McKinney did not provide enough data to permit evaluation of their estimate of different lower limiting temperatures for the populations tested. The figure cited for *punctatus* from Texas is higher than any other yet de-

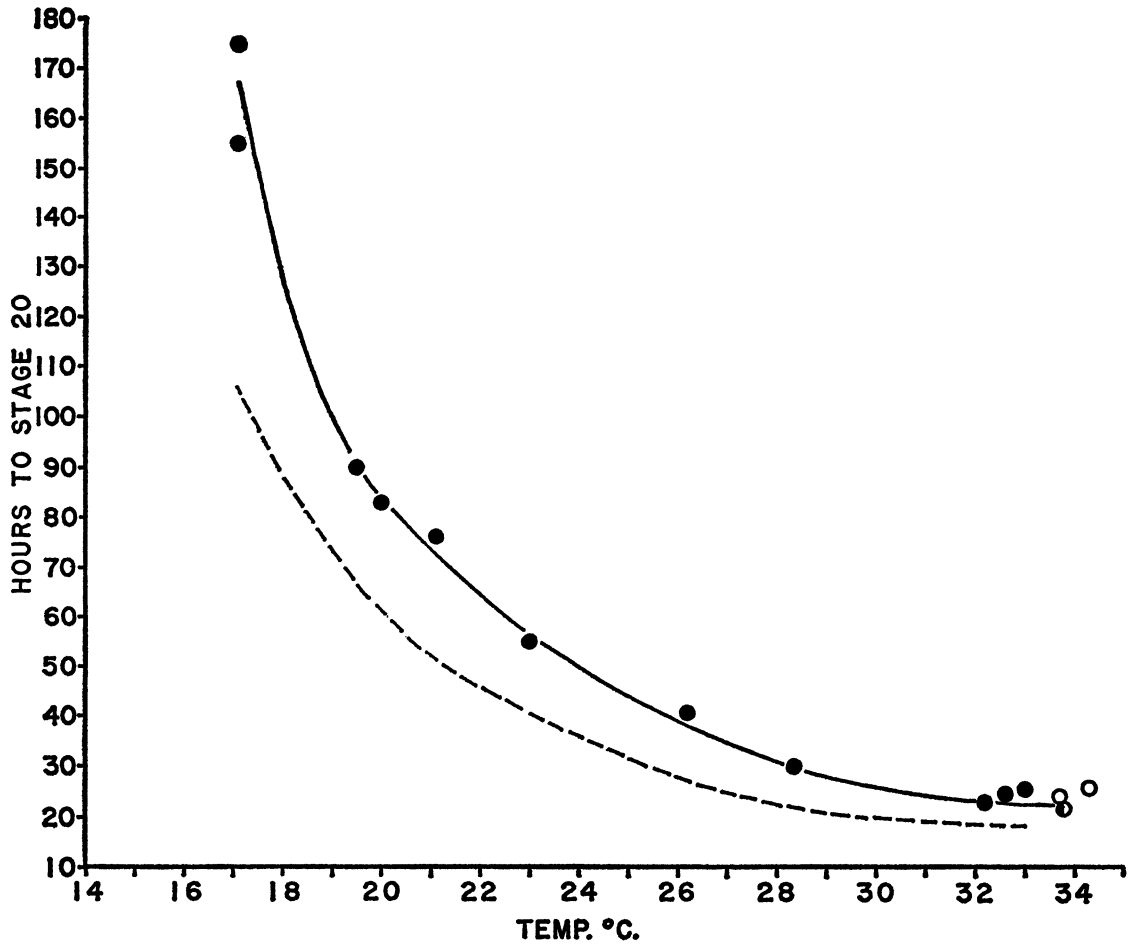


FIG. 5. Time required by embryos of *Bufo punctatus* to hatch (broken line) and to reach stage 20 (solid line) when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

Symbols: Solid symbols, normal development; open circles, less than 50 per cent survival; half-filled circle, approximately 50 per cent survival.

termined for a North American frog. The maximum is 18° C. for *Bufo valliceps* (Volpe, 1957a).

Apparently, from the data Ballinger and McKinney presented, normal development occurred at 33.4° C. Their statement (p. 23) that "the maximum lethal temperature is near 36.0°" evidently is based on the observation that at "36.0° one individual hatched while 14 stopped at gill circulation (stage 20)." Questions raised by the implication of late hatching are discussed below. It is unfortunate that so few embryos were raised, because variability from clutch to clutch at the limiting temperatures makes it necessary to have large samples.

As was the case with *Bufo cognatus*, the information on time to hatching and stage of hatching presented by Ballinger and McKinney is incompatible with that I obtained for *Bufo punctatus*. I found that hatching took place in stage 16 or, more often, in stage 17. The sentence quoted in the previous paragraph implies that most embryos concerned were still unhatched at stage 20. These authors reported a similar situation for embryos from Texas (p. 26): "Stages from early tail bud to gill circulation (17–20) were

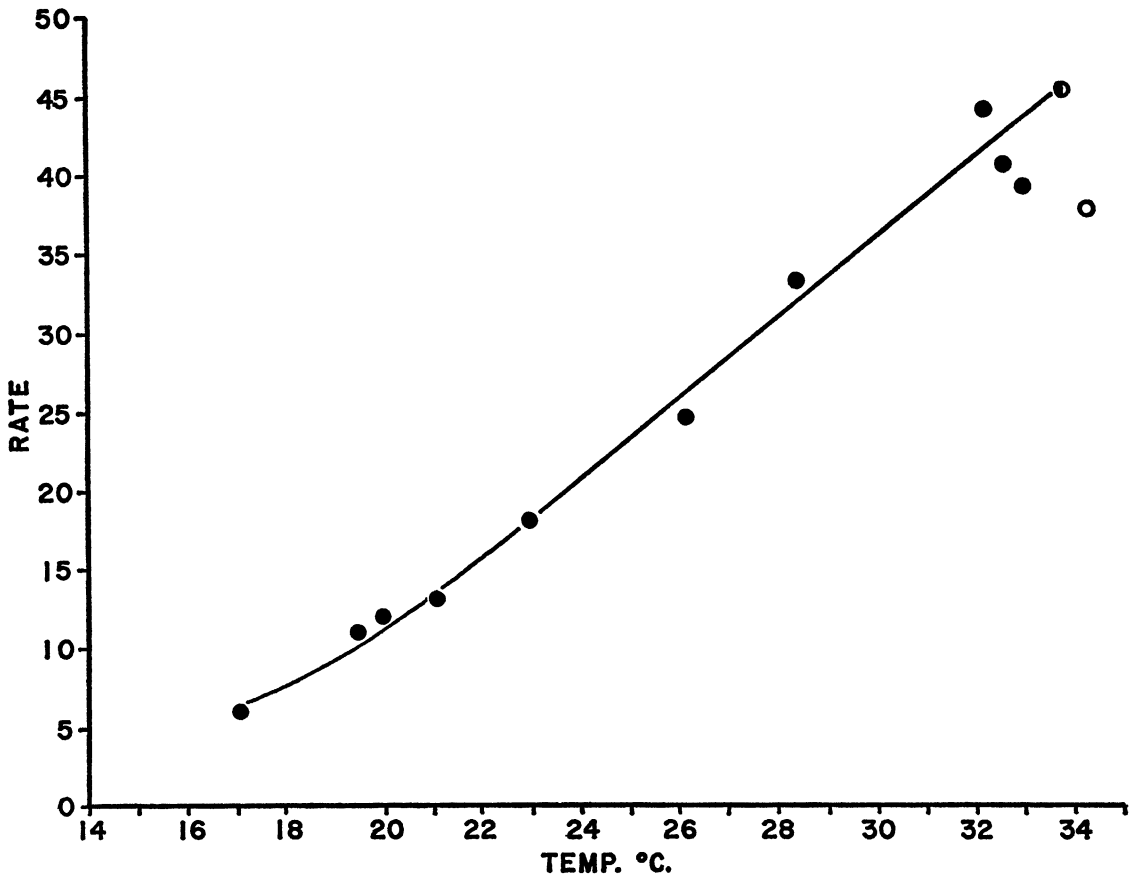


FIG. 6. Rate of development of embryos of *Bufo punctatus* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 (1/T×1000).

Symbols: Solid symbols, normal development; half-filled circle, approximately 50 per cent survival; open circle, less than 50 per cent survival.

reached at temperatures between 16.9° and 19.1°, but no individuals hatched." Data concerning time to hatching are similarly at variance. For example, Ballinger and McKinney reported: "Hatching stages were reached in 3 to 4 days [72 to 96 hours] at temperatures from 20.8° to 22.9°." In one of my experiments at 21.1° C., some had hatched by 48 hours and most by 57 hours.

Comments made in the foregoing account of *Bufo cognatus* apply here, too, and may help to explain some of the differences between my results and those of Ballinger and McKinney.

HYLA ARENICOLOR

All frogs used in the experiments came from the Chiricahua Mountains. Both artificial fertilization and natural fertilization were employed, but ovulation was induced in all instances. Implantation of two preserved pituitary glands of female *Rana pipiens* induced ovulation in from 10 to 20 hours.

LIMITING TEMPERATURES

LOWER LIMITING TEMPERATURE

The lower limit lies between 11.3° C. and 15.5° C. The embryos in one experiment at 11.3° C. reached stage 12, but none completed gastrulation, whereas in the single experi-

TABLE 4

RESULTS OF EXPERIMENTS ON *Hyla arenicolor*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
58-2	11.3	—	—	Reached stage 12 with some exogastrulation, but none survived to stage 13
58-2	15.5	196	5.1	Normal development
60-1	17.0	160	6.3	Normal development
60-1	21.2	72	13.9	Normal development
60-2	22.8	57	17.5	Normal development
58-2	24.5	52	19.2	Normal development
60-2	29.1	35	28.6	Normal development
60-1	32.9	—	—	All died in cleavage stages
60-1	34.0	—	—	All died in cleavage stages

ment at 15.5° C. there was normal development. An accurate estimate of the lower limiting temperature cannot be made with the data at hand, but it can be tentatively estimated to be 13° C.

UPPER LIMITING TEMPERATURE

Normal development occurred at 29.1 °C., but at 32.9° C. and at 34.0° C. all embryos died in cleavage stages. Therefore, the upper limit must lie well below 32.9° C.—probably not higher than 31.0° C.

EFFECT OF EXPOSURE TO HIGH TEMPERATURE IN CLEAVAGE STAGES

Forty embryos raised at room temperature were transferred to 34.8° C. when they attained stage 7. Eight hours later the embryos were in late stage 12 and early stage 13. Some were greatly exogastrulated, but others appeared normal. However, all had died within 34 hours after exposure to the higher temperature (the temperature rose 1° C. near the end of the experiment), and none developed past stage 18.

RATE OF DEVELOPMENT

Neither the lower nor upper limiting temperatures were determined precisely for this species, so the fastest and slowest possible rates of development under constant temperature conditions were not measured. At the lowest temperature at which an experiment was completed, 15.5° C., development to stage 20 took 196 hours. The most rapid development, 35 hours, occurred at 29.1° C. Extrapolation of the time-temperature curve (fig. 7) to the estimated upper limiting temperature of 31.0° C. suggests a minimum developmental time of about 32 hours.

Hatching takes place relatively late in development, typically late in stage 20.

RANA PIPIENS

All embryos used in the experiments developed from eggs collected in the Chiricahua Mountains, either at the Southwestern Research Station at an elevation of 5400 feet, or at Herb Martyr Lake 2 miles away at 5600 feet. For reasons that never were explained, I had no success in obtaining fertilizable eggs by the conventional method of inducing ovulation. Fortunately, however, I obtained some egg masses before the first cleavage had taken place and others sufficiently early in development that could be used in the experiments.

LIMITING TEMPERATURES

LOWER LIMITING TEMPERATURE

Two experiments were run at 11.1° C.: in one the eggs had developed to stage 8 before being placed at the constant temperature, and in the other to stage 9. The embryos in both experiments formed exogastrulae, and

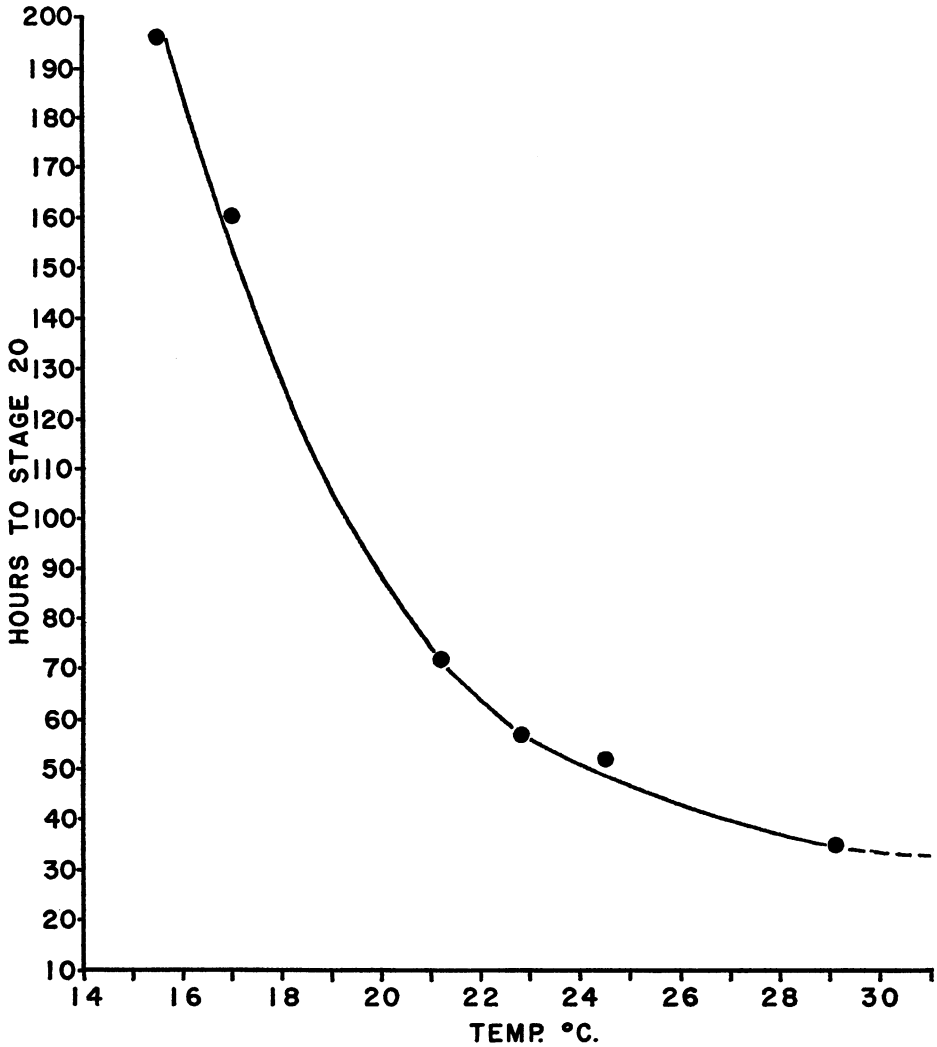


FIG. 7. Time required by embryos of *Hyla arenicolor* to reach stage 20 when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

the yolk plugs still protruded when the experiments were terminated in stages 18 and 17, respectively. The embryos might well have survived to stage 20, but the persistence of the yolk plug indicates that this temperature is below the lower limiting temperature. Also to be considered is the probability that embryos exposed to the low temperature in late cleavage stages are likely to be more resistant to cold than they would have been if exposed earlier in development. This is additional evidence that the lower limiting temperature lies above 11.1° C.

Three experiments at 15.6° C. gave normal development. In two of these the embryos were first exposed at stages 8 and 9, but in the remaining experiment the eggs were distributed before first cleavage. The lower limiting temperature evidently is not much above 11° C., and it may tentatively be set at 12° C.

Upper Limiting Temperature

In one experiment at 34.5° C. no embryo survived beyond late stage 9. At 33.3° C. development proceeded further, but many of

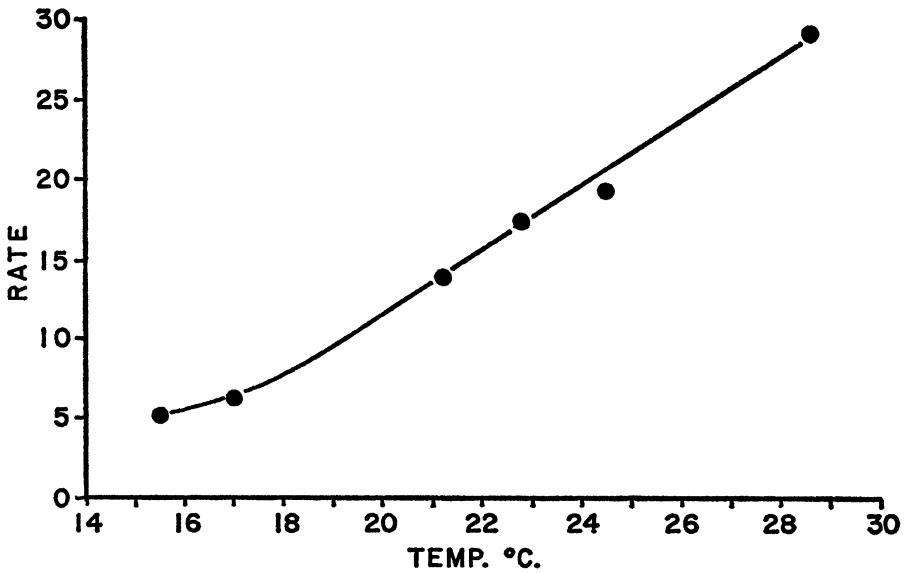


FIG. 8. Rate of development of embryos of *Hyla arenicolor* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 (1/T×1000).

the embryos (which were started at stage 6) died before completing gastrulation, all survivors were exogastrulated, and none survived to a recognizable stage 20. The embryos in an experiment at 31.5° C. developed normally except that the initiation of gill circulation was retarded. This temperature may be regarded as approximating the upper limit for normal development.

EFFECT OF EXPOSURE TO HIGH TEMPERATURE IN CLEAVAGE AND POSTGASTRULA STAGES

Embryos in early stage 8 were placed in water at 34.4° C. Between three and seven hours later the temperature dropped slightly and then remained at 33.6° to 33.8° C. Many embryos died in the pregastrula stages, and none survived beyond stage 19. Concurrently, groups of embryos from the same egg mass were raised at 32.5° C. and 32.0° C. In marked contrast these embryos underwent normal development.

Three additional groups of embryos from the same egg mass were allowed to develop to early stage 11 before being placed at elevated temperatures. As would be expected from the results of the other experiments, those raised at the two lower temperatures (32.0° and 32.5° C.) developed normally. Although most embryos raised at 33.6° to 33.8° C. failed to hatch, many did survive posthatching. These showed some abnormality in having the back slightly arched.

One group of embryos in stage 13 was placed in water at 34.4° to 34.7° C. Many of these continued to develop to what appeared to be stage 19 (the marker of this stage, heartbeat, was not noted), but most died without hatching, and the few that survived to hatch had warped bodies and lacked gill circulation.

Evidently by the time the embryos develop to stage 8 they can tolerate a constant temperature of 32.5° C., which is 1° above the maximum that could be tolerated earlier in cleavage. If permitted to develop to stage 11 at normal temperatures before being subjected to the higher temperature, at least some embryos survive a constant temperature of 33.6° to 33.8° C., even though this temperature range is intolerable to those embryos that have developed only to stage 8.

RATE OF DEVELOPMENT

Because eggs in some masses had undergone several cleavages before being distributed among the constant-temperature baths, it was necessary, in these instances, to estimate

TABLE 5

RESULTS OF EXPERIMENTS ON *Rana pipiens*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours[a]	Rate, 1000/Time	Comments
58-1	11.1	—	—	Started in stage 9; exogastrulation persisted until termination of experiment in stage 17 when most embryos were dead
58-2	11.1	—	—	Started in stage 8; exogastrulation persisted in some embryos into stage 16; most embryos alive at termination of experiment in stage 17
58-2	15.6	226	4.4	Started in stage 8; normal development
58-3	15.6	225	4.4	Normal development
—	17.9	149	6.7	Data furnished by R. Ruibal
58-4	18.1	155	6.4	Started in stage 6; normal development
58-5	18.1	161	6.2	Started in stage 4; normal development
58-3	20.4	130	7.7	Normal development
58-2	24.6	81	11.8	Started in stage 8; normal development
58-6	26.8	69	14.3	Started in stage 4; normal development
58-4	27.1	67	14.8	Started in stage 6; normal development
58-6	29.0	67	14.9	Started in stage 4; normal development
58-3	29.5	57	17.5	Normal development, virtually 100 per cent survival
58-5	31.5	—	—	Started in stage 4; time to stage 20 not determined because of delay in attainment of gill circulation, development otherwise normal
58-4	33.3	—	—	Started in stage 6; some died in cleavage stages, others as exogastrulae; none survived to normal stage 20, though some hatched
58-5	34.5	—	—	Started in stage 4; none lived beyond stage 9

[a]In experiments started after cleavage had commenced, the time required to reach the starting stage was estimated and is included in the figure given.

the time that it would have taken the embryos to reach the stage at which they were distributed and to compute the total time accordingly. The best experiment was 58-3, in which the eggs were distributed before the first cleavage had taken place. The time-temperature curve (fig. 9) is drawn with particular reference to this experiment, but most of the others conform fairly well. The embryos of experiment 58-6 at 29.0° C. seemed to develop normally but took unusually long to develop gill circulation, possibly a high-temperature effect.

The slowest development was at 15.5° C. One group of embryos reached stage 20 in 225 hours, whereas two others took 250 to 252 hours, but this temperature is probably at least 3° above the minimum for normal development. If the slope of the rate curve (fig. 10) is approximately accurate, development could take as long as 330 hours at 14° C. At 29.5° C. stage 20 was reached in 57 hours. This temperature is probably within 2° of the maximum for normal development, and the most rapid development probably would be no more than three or four hours less than observed at 29.5° C.

Hatching may begin as soon as early stage 19, but it commonly begins late in this stage or early in stage 20.

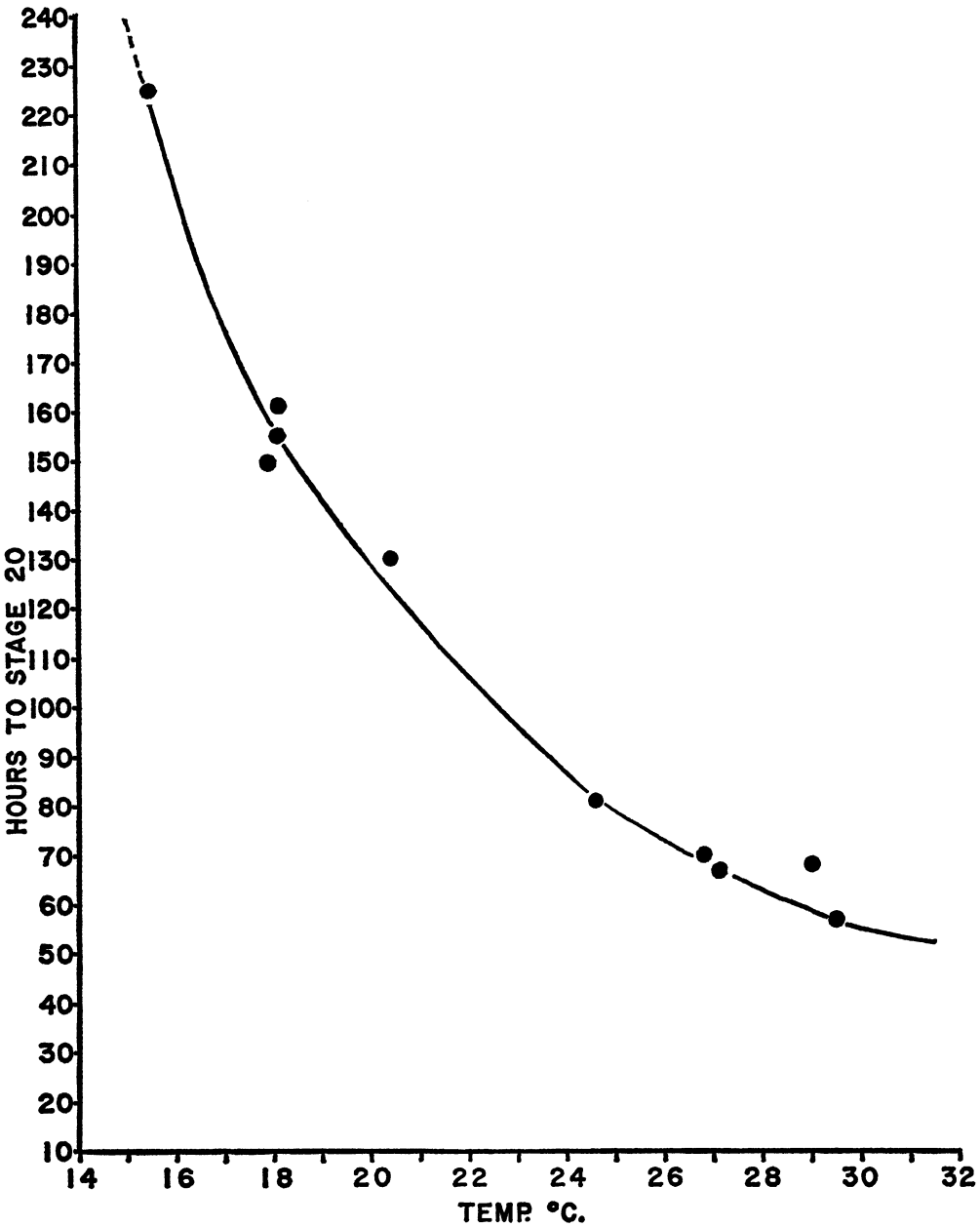


FIG. 9. Time required by embryos of *Rana pipiens* to reach stage 20 when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

COMPARISON WITH PREVIOUSLY PUBLISHED RESULTS

Rana pipiens is noteworthy for being the only species of anuran known to have considerable geographic variation in embryonic temperature tolerance and rate of development. The basic work on *pipiens* was done by Moore (1949a), who experimented on eggs obtained from frogs collected at several localities ranging from Quebec and Wisconsin

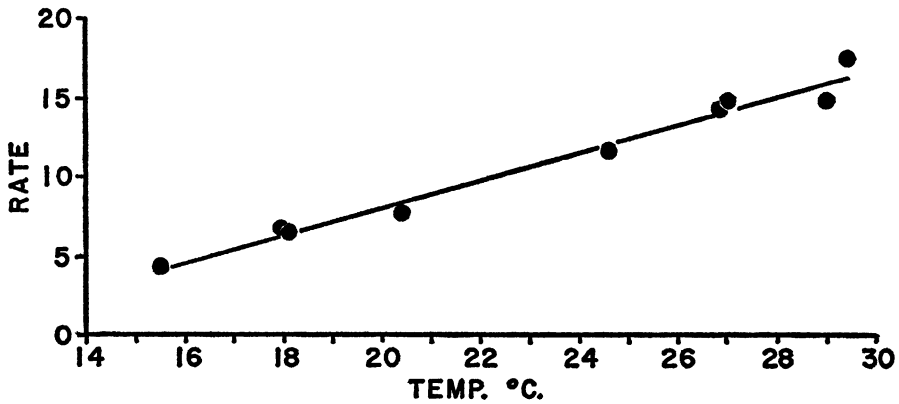


FIG. 10. Rate of development of embryos of *Rana pipiens* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 (1/T×1000).

to western Texas and San Luis Potosí, Mexico. Volpe (1954) and Ruibal (1955) added information on other Mexican populations, Volpe (1957b) studied *pipiens* in Costa Rica, and Ruibal (1962) reported on a population in southern California.

The limiting embryonic temperatures for the species as a whole range from 5° to 35° C., but no one population exhibits such a wide range (table 6). The lower limiting temperature of the Arizona sample is as high as has previously been recorded, whereas the upper limiting temperature is in the middle of the range of values previously recorded. The range of 12° C. to 31.5° C. seen in the Arizona sample is not exactly duplicated in other samples, but is closely approached by the embryos from California (11° to 29° C.) and San Luis Potosí (12° to 33° C.). The embryos showing the narrowest span of tolerance, 18° C., are those from California, but this range is closely approached by those from Arizona at 19° C.

Embryos of *Rana pipiens* from different geographic regions may have different rates of development. At temperatures below about 18° C. embryos from Vermont and other northern localities develop more rapidly than any others tested, whereas at higher temperatures the developmental rate of the Vermont embryos is exceeded by that of embryos from such warm, lowland localities as San Felipe Creek, California; San Diego, Puebla, Mexico; and Ocala, Florida (fig. 11).

Embryos from Arizona are notable for having the slowest rate of development at all temperatures. The closest approach is seen in embryos from southern California (Ruibal, 1962) and Zempoala in the highlands (elevation 9840 feet) near Mexico City (Ruibal, 1955). The time-temperature curve for Zempoala embryos virtually duplicates that given for the sample from southern California (fig. 11).

Ruibal (1962) showed that the various populations of *Rana pipiens* could be placed in three major categories, using as criteria the relative rates of embryonic development at high and low temperatures, embryonic temperature tolerance, and degree of hybrid inviability when crossed with other populations of *pipiens*. Ruibal distinguished the "cold" races as those with rapid development at low temperatures, tolerance of low temperatures, and considerable hybrid inviability when crossed with "warm" races. The "warm" races show rapid development at high temperatures, tolerance to high temperatures, and considerable hybrid inviability when crossed with "cold" races. The "slow" races show slow development at all temperatures, a narrow range of temperature tolerance, and relatively little hybrid inviability when crossed with either "warm" or "cold" races.

The "cold" races are found from Colorado to eastern Canada, Vermont, and New Jersey. The "warm" races inhabit lowland areas from Florida to eastern Mexico. The "slow" races occur in southern California, at high and intermediate elevations in Mexico, and in the highlands of Costa Rica.

TABLE 6

TEMPERATURE RANGE FOR NORMAL EMBRYONIC DEVELOPMENT OF *Rana pipiens* FROM NINE GEOGRAPHIC REGIONS

Area	Range in Degrees Centigrade	Span in Degrees Centigrade	Authority
Northeastern United States and Canada	5°–28°	23°	Moore, 1949a
Louisiana	5°–32°	27°	Moore, 1949a
Florida (Ocala)	9°–33°	24°	Moore, 1949a
Texas (western)	10°–32°	22°	Moore, 1949a
Florida (Englewood)	11°–35°	24°	Moore, 1949a
California (San Felipe Creek)	11°–29°	18°	Ruibal, 1962
Arizona (Chiricahua Mts.)	12°–31.5°	19°	Present work
Mexico (Axtla, San Luis Potosí)	12°–33°	21°	Moore, 1949a

The individuals of *pipiens* from the Chiricahua Mountains clearly fit into the "slow" group, judged by the two criteria of rate of development and range of temperature tolerance. Ruibal (*in litt.*) reports that eggs from North Dakota fertilized by sperm from a male from the Chiricahua Mountains produced normal embryos. The cross of North Dakota×North Dakota produced embryos which (at 17.9° C). developed at the rate characteristic of the "cold" races. Therefore, the Chiricahua population satisfies the criterion of hybridization as well.

Other than to note the evident affinity of the Arizona frogs with those of southern California and the highlands of Mexico, it is inadvisable to speculate further on relationships. Although it has been customary in recent years to recognize one species of leopard frog, with three races (e.g., Conant, 1958), virtually everyone familiar with the frogs agrees that such a taxonomic arrangement does not do justice to the morphological and physiological diversity of the natural populations. Studies under way by other workers may show that more than one species currently passes under the name *pipiens*. For example, M. J. Littlejohn (personal communication) finds differences between the mating calls of populations of *pipiens* as great as those of unquestionably good species, and in Colorado populations of two distinct but allopatric forms of *pipiens* exist within 1 mile of each other without evidence of intergradation (Post and Pettus, 1966).

SCAPHIOPUS BOMBIFRONS

The adults were collected in San Simon Valley near Portal, Arizona, and Rodeo, New Mexico, and in Animas Valley, New Mexico. In four instances pairs found in amplexus in breeding congresses were allowed to remain in amplexus until ovulation took place, whereas at two other times ovulation was induced by injection of fresh pituitary glands of female *Scaphiopus bombifrons*. In one instance injection of two glands induced ovulation between 10 and 17 hours later.

Scaphiopus bombifrons and *S. hammondii* are known to hybridize in the region where I collected my experimental animals (Bogert, 1960, pp. 285–287). From the physical characteristics and, in some instances, the mating calls of the individuals used in my experiments, I am confident that no hybrids were involved. The individuals used were preserved and are catalogued in the collection of the American Museum of Natural History, where they may be examined. In the following paragraph, the number of the experiment is given first, followed in parentheses by the catalogue numbers of the individuals that provided eggs and sperm.

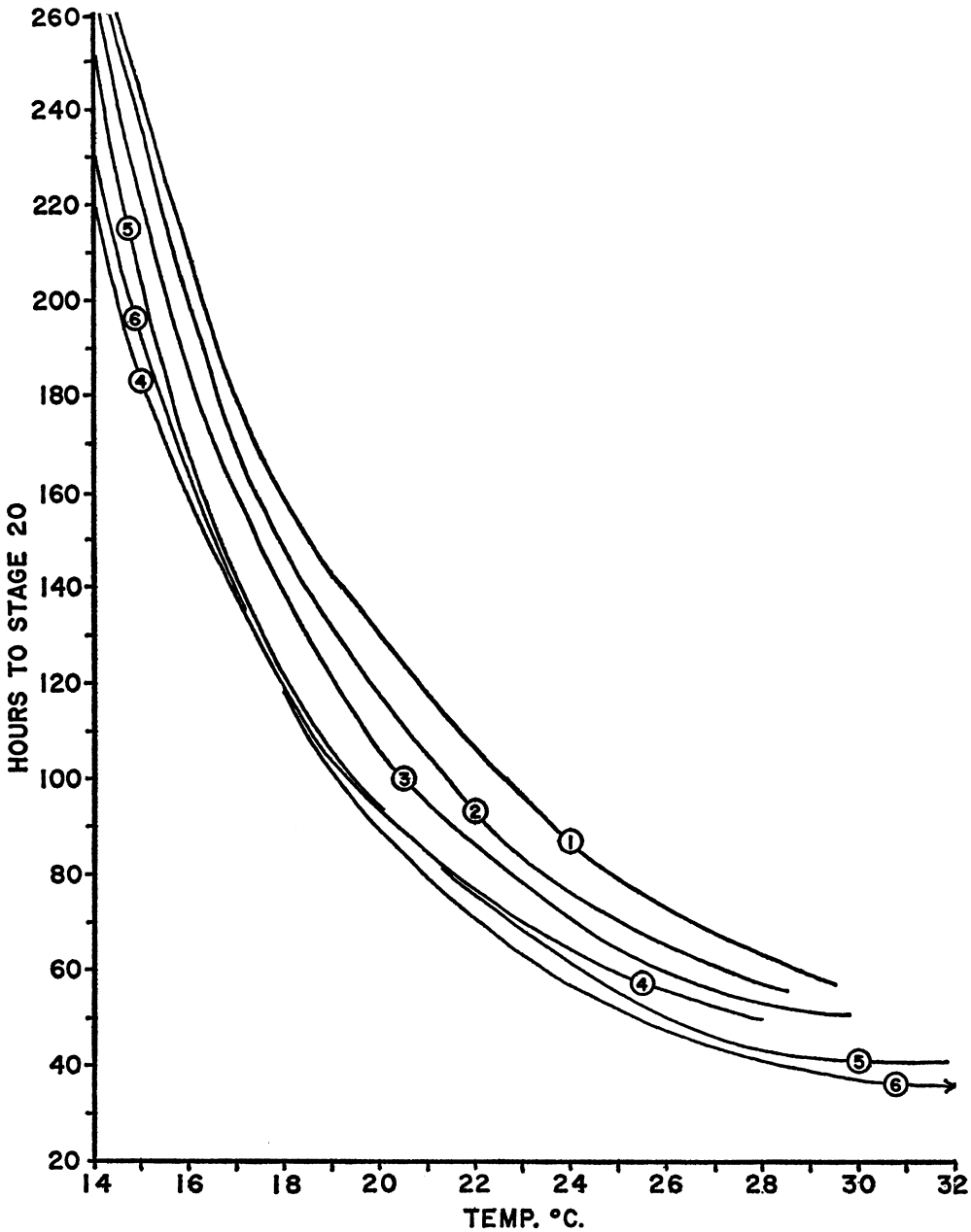


FIG. 11. Time required by embryos of various populations of *Rana pipiens* to reach stage 20 at constant temperatures. 1. Chiricahua Mountains, Arizona. 2. Southern California (Ruibal, 1962). 3. Costa Rica (Volpe, 1957b). 4. Vermont (Ruibal, 1962). 5. San Diego, Puebla, Mexico (Ruibal, 1955). 6. Ocala, Florida (Moore, 1949a).

Scaphiopus bombifrons

58-1 (A.M.N.H. Nos. 62461, 62462)
58-2 (A.M.N.H. Nos. 62463, 62464)
58-4 (A.M.N.H. Nos. 62738, 62739)
58-5 (A.M.N.H. Nos. 62740, 62742)
60-1 (A.M.N.H. Nos. 65722, 65723)
60-2 (A.M.N.H. Nos. 65724, 65725)

Scaphiopus hammondii

58-1 (A.M.N.H. Nos. 62772, 62773)
58-2 (A.M.N.H. Nos. 62774, 62775)
58-3 (A.M.N.H. Nos. 62776, 62777)
58-5 (A.M.N.H. Nos. 62778, 62779)
58-6 (A.M.N.H. Nos. 62791, 62792)
60-1 (A.M.N.H. Nos. 65734, 65735)
60-2 (A.M.N.H. Nos. 65736, 65737)
60-3 (A.M.N.H. Nos. 65738, 65739)

LIMITING TEMPERATURES

Lower Limiting Temperatures

Development was abnormal at 11.5° C.; in two experiments the embryos suffered much exogastrulation, and none reached stage 20 in normal condition. In a single experiment at 15.5° C. development was normal. Consideration of the degree of abnormality exhibited at 11.5° C. leads me to estimate that the lower limiting temperature is about 13° C.

Upper Limiting Temperature

High mortality was evident in four experiments conducted at 33.2°, 34.0°, 34.2°, and 34.5° C. Approximately 50 per cent of the embryos in one experiment at 33.0° C. achieved stage 20 in normal condition, but in another experiment at the same temperature most died in the course of gastrulation. Evidently this temperature is quite close to the upper limiting temperature, which I estimate to be 32.5° C.

Effect of Exposure to High Temperature in Cleavage Stages

Embryos raised to stage 7 at room temperature were placed in a water bath at 34.6° C. These developed normally and were active and evidently healthy in stage 23 when the experiment was terminated.

TABLE 7

Results of Experiments on *Scaphiopus bombifrons*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
58-1	11.5	—	—	Severe exogastrulation; none reached normal stage 20
58-2	11.5	—	—	Much exogastrulation; some developed to stage 17
58-2	15.5	123	8.1	Normal development
60-1	17.1	75	13.3	Normal development
58-4	18.2	70	14.3	Normal development
58-5	18.2	70	14.3	Low survival not attributable to temperature effect
60-1	21.2	41	24.4	Normal development
58-2	24.6	30	33.3	Normal development
58-4	27.3	27	37.0	Normal development
60-1	33.0	19.5	51.3	30 of 61 (50%) survived to stage 20
60-2	33.0	—	—	18 of 87 (21%) survived to stage 20; remainder died in gastrulation
58-4	33.2	20	50.0	Development normal, but gill development delayed and much late mortality
60-2	34.0	—	—	All died in or before stage 12
60-1	34.2	—	—	Almost 100 per cent mortality in cleavage stages; none survived past stage 12
60-2	34.5	—	—	All died in or before stage 12

RATE OF DEVELOPMENT

Development to stage 20 took 123 hours at 15.5° C. The lower limit of temperature tolerance was not determined, but the trend of the rate curve (fig. 13) indicates that lowering the temperature by as little as 1° to 14.5° C. would add about 60 hours to the development time. At 33° C. surviving embryos reached stage 20 in 19.5 hours. This temperature evidently is slightly above the upper limit for normal development, but a drop of one-half degree would make little difference in the rate.

Hatching may take place late in stage 19 or in stage 20. Commonly, some embryos in a clutch hatched in the earlier stage, whereas others in the same group were delayed although were in no obvious way abnormal.

COMPARISON WITH PREVIOUSLY PUBLISHED WORK

There is no information in the literature on the temperature tolerance of embryos of *S. bombifrons*, but Trowbridge (1942) discussed the rate of development at 23° to 25° C. of embryos from Oklahoma. "Stage 19" was reached at 28 hours; "stage 20," at 30.5 hours. Stage 20 of Trowbridge is not exactly equivalent to stage 20 as used here, for Trowbridge stated that gill circulation (the criterion of stage 20) became evident during (but not at the beginning of) stage 19. The variation of 2° C. in temperature makes exact comparison impossible, but the rate found by Trowbridge evidently is closely similar to that of my sample in which embryos at 24.6° C. reached stage 20 in approximately 30 hours.

SCAPHIOPUS COUCHII

Scaphiopus couchii was the only species tested in which all experimental animals did not come from the same general region in southeastern Arizona and adjacent New

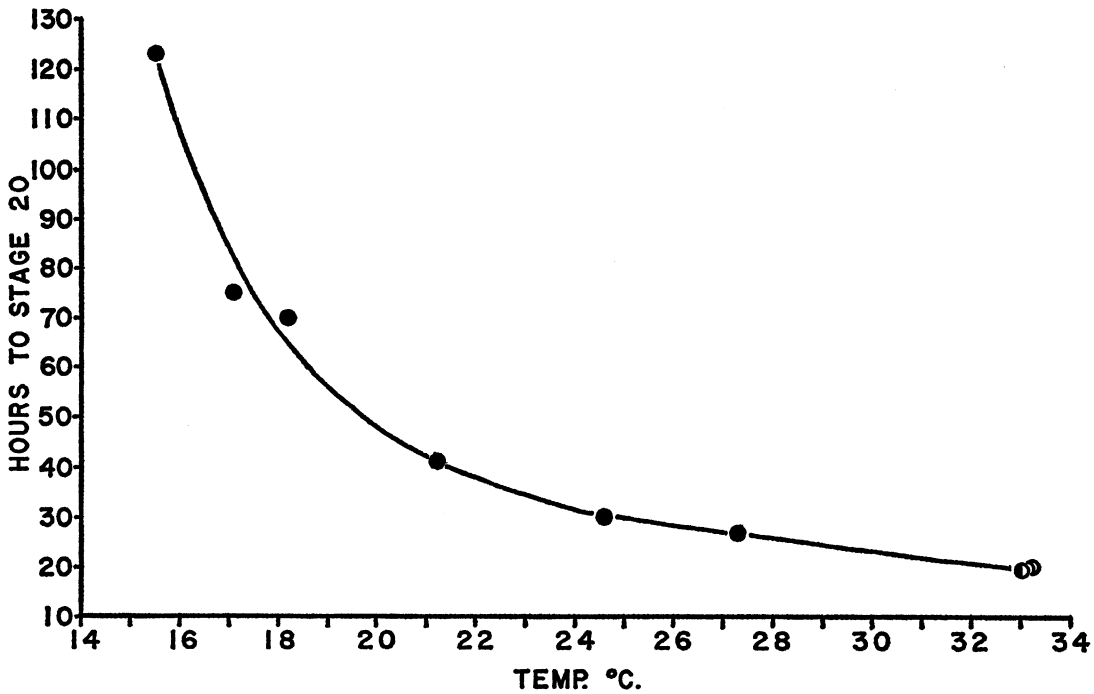


FIG. 12. Time required by embryos of *Scaphiopus bombifrons* to reach stage 20 when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

Symbols: Solid symbols, normal development; open circle, less than 50 per cent survival; half-filled circle, approximately 50 per cent survival.

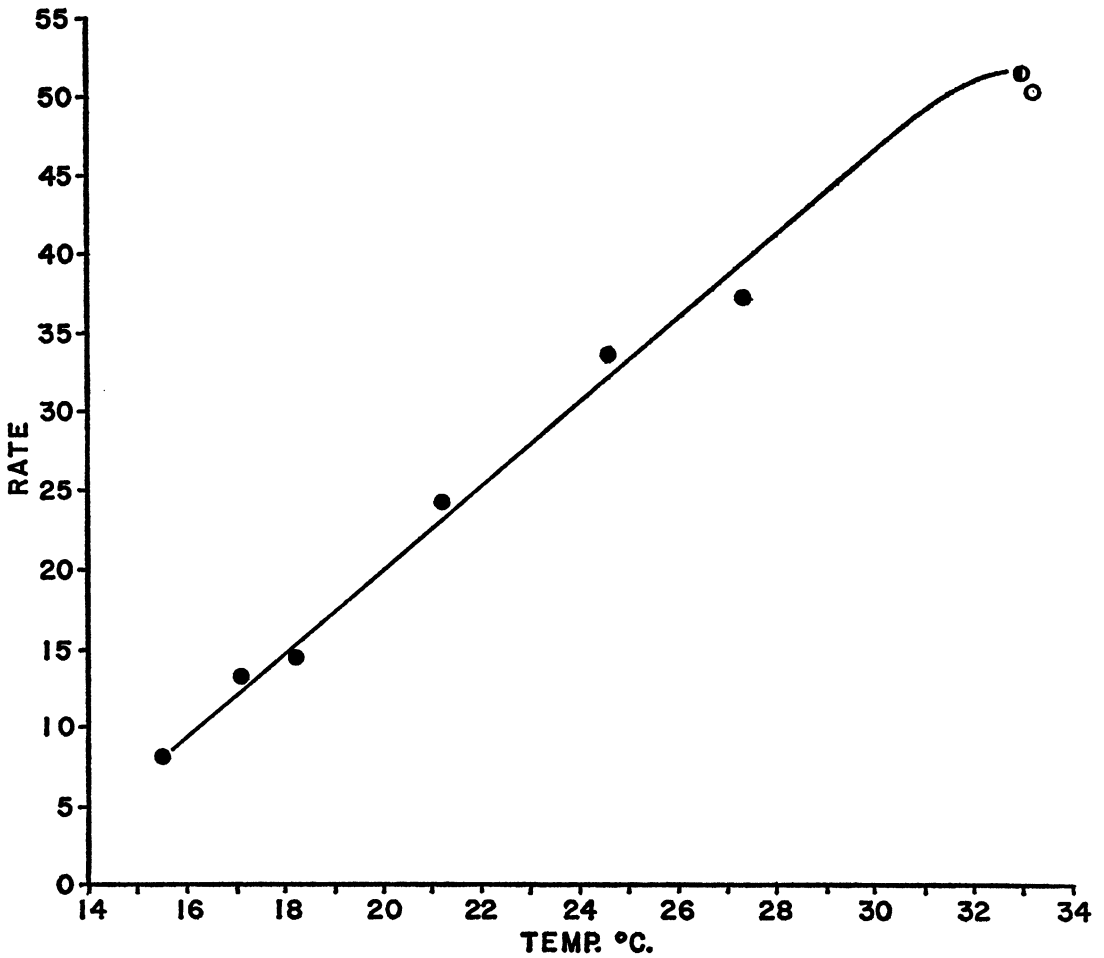


FIG. 13. Rate of development of embryos of *Scaphiopus bombifrons* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 ($1/T \times 1000$).

Symbols: Solid symbols, normal development; open circle, less than 50 per cent survival; half-filled circle, approximately 50 per cent survival.

Mexico. The animals used in most experiments were collected in San Simon Valley (in the vicinity of Portal and Rodeo) and Animas Valley (Road Forks), but in two experiments I utilized females captured at Hermosillo, Sonora, Mexico, and another female came from Guaymas, Sonora. The males in these three experiments were from Hermosillo. Hermosillo is about 225 miles southwest of the study area in Arizona-New Mexico, and Guaymas is 300 miles south-southwest.

In some of the experiments females in amplexus were permitted to ovulate without additional stimulation, but injection of preserved pituitary glands of *S. couchii* was utilized in most instances. Three or four glands usually induced ovulation in from six to nine hours; the shortest period was about four hours, the longest was about 20.

LIMITING TEMPERATURES

Lower Limiting Temperature

At 11.5° C. in two experiments there were only a few irregular cleavages. In one experiment at 13.6° C. early development was relatively normal, and gastrulation took place with little exogastrulation. Abnormalities, however, became pronounced later in development, and the few larvae that hatched were highly abnormal. They were edematous, and

TABLE 8

RESULTS OF EXPERIMENTS ON *Scaphiopus couchii*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
58-1	11.5	—	—	No development
58-2	11.5	—	—	No development
60-4	13.6	—	—	A few abnormal embryos hatched
58-1	15.5	120	8.3	Normal development
58-2	15.5	148	6.8	Many embryos deformed and dead
58-6	15.5	133	7.5	Normal development, but some mortality
60-1	17.0	91	11.0	Normal development
58-10	18.1	74	13.5	Normal development
58-11	18.1	75	13.3	70 of 97 (72%) normal development
58-13	18.2	74	13.5	Abnormal mortality not due to temperature
58-14	18.2	70	14.3	Hatching delayed
60-4	20.1	52	19.2	Normal development
58-4	20.3	56	17.8	Normal development
58-5	20.5	59	16.9	High mortality not due to temperature
58-7	21.0	57.5	17.4	Normal development (parents from Sonora, Mexico)
58-9	21.0	56	17.8	Abnormal mortality not due to temperature (parents from Sonora, Mexico)
58-6	21.1	48	20.8	Normal development
60-1	21.3	45.5	22.0	Normal development
60-2	23.1	36	27.8	Normal development
60-3	23.1	37	27.0	Normal development
58-1	24.5	28.5	35.1	Normal development
58-2	24.5	28	35.7	Normal development
58-3	24.5	28	35.7	Abnormal mortality not due to temperature
60-4	26.2	25	40.0	Normal development
58-14	26.5	24	41.7	Normal development
58-10	27.0	22.5	44.4	Normal development
58-11	27.1	22.5	44.4	Normal development
60-2	28.4	22	45.5	Normal development
60-3	28.4	22	45.5	Normal development
58-4	29.5	20	50.0	Abnormal mortality not due to temperature
58-5	29.5	20.5	48.8	Abnormal mortality not due to temperature
58-6	29.5	20.5	48.8	Normal development
58-14	31.4	18	55.5	Normal development
58-12	31.7	15.5	64.5	Normal development
60-4	32.0	16.5	60.6	Development essentially normal; 10 of 48 (20%) slightly abnormal
60-2	32.2	16	62.5	Normal development
60-3	32.2	16	62.5	Normal development
60-1	33.1	16	62.5	81 of 112 (72%) developed normally
60-4	33.1	—	—	Development essentially normal, but end point not observed; 6 of 39 (15%) slightly abnormal

TABLE 8—(*Continued*)

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
58-10	33.3	16.5	60.6	74 of 80 (92%) developed normally
60-4	33.5	—	—	Development essentially normal, but end point not observed; 9 of 48 (19%) slightly abnormal
58-11	33.6	15	66.7	Normal development
60-2	33.6	15.5	64.5	Normal development
60-3	33.6	15.5	64.5	Normal development with slight early mortality
58-12	33.9	15.5	64.5	Normal development
60-1	34.1	15	66.7	53 of 141 (38%) developed normally
60-4	34.2	—	—	Development largely normal, but end point not observed; 14 of 47 (30%) abnormal development
58-9	34.2	14	71.4	High mortality (parents from Sonora, Mexico)
58-7	35.2	—	—	45 of 50 (90%) died in or before stage 12, none survived to normal stage 20 (parents from Sonora, Mexico)
58-8	35.5	—	—	No normal development
60-2	35.5	—	—	Most died in cleavage stages, 3 of 62 (5%) reached abnormal stage 20

circulation was not established in the abnormally small gills. Three experiments at 15.6° C. produced variable results, but there is reason to suspect that pollution may have been responsible for mortality in the two experiments that showed numerous dead embryos. One experiment at this temperature gave normal development, with high survival. The lower limiting temperature probably is close to 15.5° C.

Upper Limiting Temperature

In all experiments up to 33.9° C. there was a high rate of survival, but in one experiment at 34.1° only 38 per cent of 141 embryos reached stage 20 in apparently normal condition; the remainder were either dead or grossly deformed. Almost all embryos in one experiment at 34.2° C. died, whereas in another most developed normally. In one experiment at 35.2° and in two at 35.5° all embryos died before hatching. Evidently the upper limiting temperature is approximately 34° C.

Effect of Exposure to High Temperature in Cleavage Stages

Embryos in stage 8 were placed in water at 34.9° C. The temperature increased within four hours to 35.8° C. and thereafter remained at that level. Gastrulation and further development were completely normal.

RATE OF DEVELOPMENT

At the lower limiting temperature of 15.5° C. development to stage 20 took 120 to 148 hours. There is reason to suspect that pollution was responsible for prolonging the period to 148 hours in one experiment. The shortest period of development was 14 hours at 34.2° C. Most of the embryos in this group died, however, and the shortest period within the range of normal development was 15 hours at 33.6° C.

In several experiments all embryos hatched in stage 18, whereas in others some were delayed into stage 19. Exceptionally, hatching did not take place until stage 20. The "hatch-

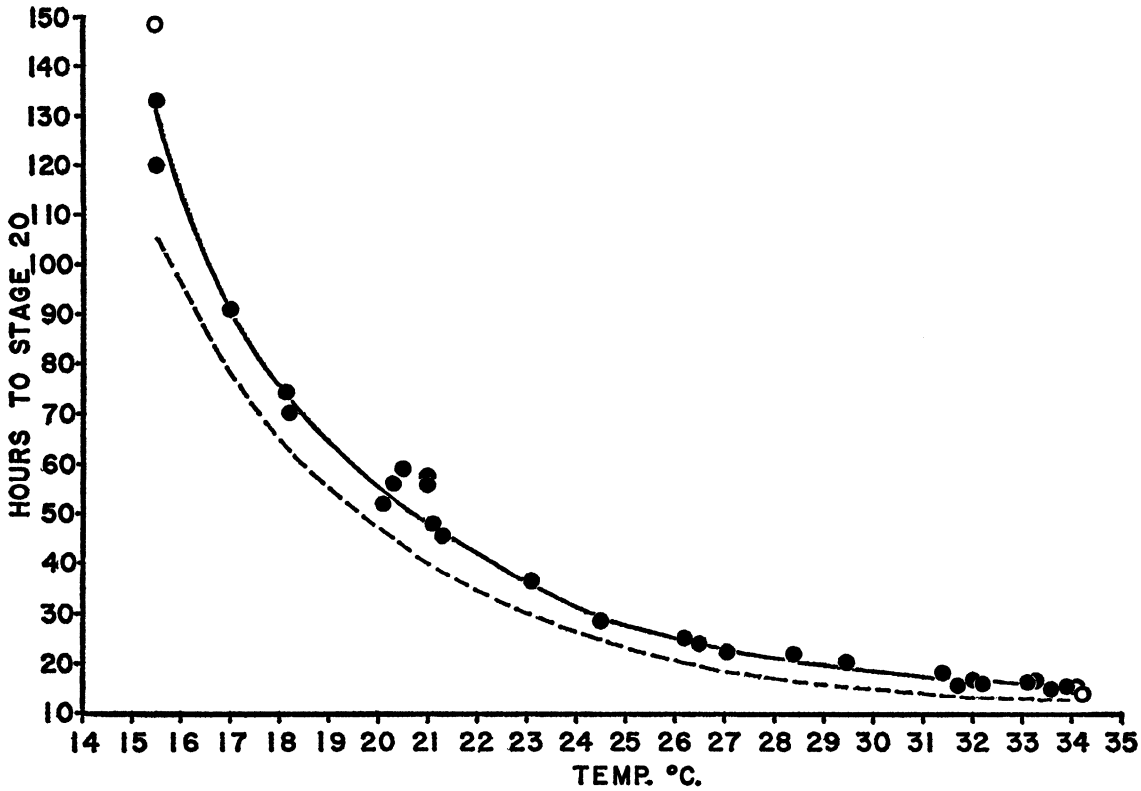


FIG. 14. Time required by embryos of *Scaphiopus couchii* to hatch (broken line) and to reach stage 20 (solid line) when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

Symbols: Solid symbols, normal development; open circles, less than 50 per cent survival.

ing" curve in figure 14 is an estimate of the time at which most embryos may be expected to have hatched. The exceptionally rapid development of this species is apparent in one of the experiments at 33.6° C., in which all embryos had hatched in stage 18 by 12 hours and stage 20 was reached in 15 hours.

COMPARISON WITH PREVIOUSLY PUBLISHED WORK

Hubbs and Armstrong (1961) raised embryos resulting from one cross of *S. couchii* at several relatively constant temperatures between 10° and 28° C. They reported (1961, p. 359) that embryos "exposed to temperatures less than 15° C. did not hatch, whereas those exposed to temperatures more than 16° C. did." This result is in good agreement with my determination of 15.5° as the lower limiting temperature, and indicates that the lower limiting temperature is the same for populations in my study area as it is for those at Austin, Texas, about 650 miles to the east. The highest temperature used by Hubbs and Armstrong, 28° C., is well below the upper limiting temperature. Hubbs and Armstrong discussed the rate of development only in terms of days to hatching, so the data are not refined enough to be used comparatively.

Ballinger and McKinney (1966, p. 26) provided additional confirmatory evidence relating to the lower limiting temperature. These authors worked with material from the same general region of Texas as did Hubbs and Armstrong. They reported: "Eggs exposed to 14.2° reached early tail bud (stage 17), but failed to hatch. All eggs exposed to temperatures greater than 15.0° hatched." The highest temperature they used was 28.4° C.

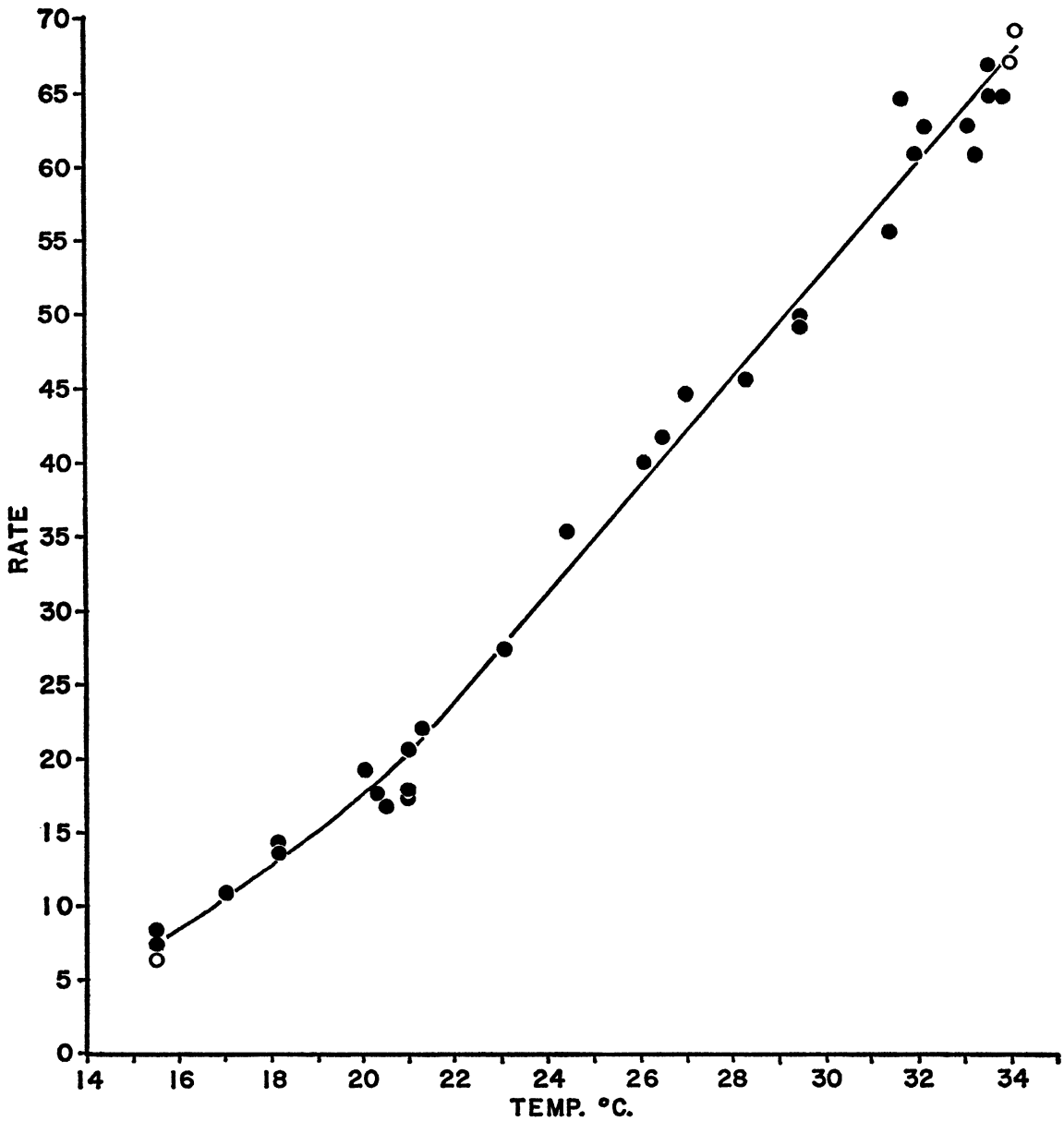


FIG. 15. Rate of development of embryos of *Scaphiopus couchii* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 ($1/T \times 1000$).

Symbols: Solid symbols, normal development; open circles, less than 50 per cent survival.

SCAPHIOPUS HAMMONDII

All experimental animals came from the vicinity of Portal, either from a ranch at Portal or from a tank 2 miles north-northwest. In one instance a female found in amplexus in a breeding chorus was injected with two female and one male *hammondii* pituitaries after having remained in amplexus for three hours. She commenced oviposition four hours after injection. Another female captured in amplexus remained

in amplexus for 13 hours and oviposited only five and one-half hours after receiving an injection of two female *hammondii* pituitaries. In four other instances injection of two pituitaries into frogs not in amplexus when captured produced ovulation in from seven to 11 hours.

LIMITING TEMPERATURES

Lower Limiting Temperature

The lower limiting temperature for this species was not established, though it evidently lies well below 15.6° C., for three experiments at this temperature gave entirely normal development.

Upper Limiting Temperature

In experiments conducted at 33.0°, 33.5°, 33.8°, 34.1°, 35.0°, and 35.5° C. development was highly abnormal. Even at the lowest temperature cited almost all embryos died and only a few survived, exogastrulated, even as far as stage 17. In marked contrast, the embryos in one experiment at 32.2° C. developed normally. The upper limiting temperature must be approximately 32.5° C.

Effect of Exposure to High Temperature in Cleavage Stages

Groups of embryos from the same clutch were placed in water at approximately 35.2° C. successively as they reached stages 3, 4, 5, 6, and 7. Those exposed in stages 3, 4, and 5 died without further development. Those in stage 6 reached stage 9 before dying, and those in stage 7 survived to stage 16, but all died.

TABLE 9

Results of Experiments on *Scaphiopus hammondii*

Experiment Number	Temperature in Degrees Centigrade	Time to Stage 20 in Hours	Rate, 1000/Time	Comments
58-1	15.6	99	10.1	Normal development
58-2	15.6	98.5	10.1	Normal development
58-3	15.6	95	10.5	Normal development
60-2	17.3	81	12.3	Normal development
58-6	18.3	72	13.9	Normal development
60-2	21.3	42	23.8	Normal development
60-3	22.7	34	29.4	Normal development
58-1	24.6	28.5	35.1	Normal development
58-2	24.6	32	31.3	Normal development
58-3	24.6	29	34.5	Normal development
58-5	24.6	31	32.2	Normal development
58-6	26.6	29	34.5	Normal development
58-1	31.5	20	50.0	High mortality in one tray, low in a replicate; probably not a temperature effect
58-6	31.5	21	47.6	Normal development
60-3	32.2	18	55.6	Normal development
60-2	33.0	—	—	Most died before stage 12, a few survived to abnormal stage 17
58-5	33.5	—	—	Much exogastrulation, no survival past stage 17
58-3	33.8	17	58.8	More than 50 per cent mortality
60-2	34.1	—	—	All died in cleavage stages
60-2	35.0	—	—	All died in early cleavage
58-2	35.5	20	50.0	More than 50 per cent mortality; low rate due to delay in establishment of gill circulation

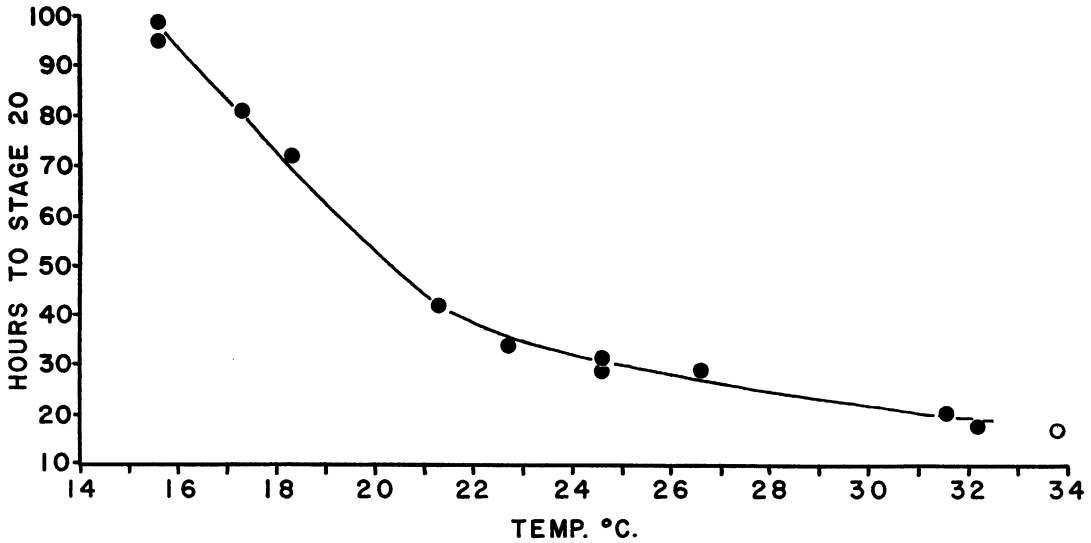


FIG. 16. Time required by embryos of *Scaphiopus hammondii* to reach stage 20 when incubated at constant temperatures. Symbols mark times at which approximately 50 per cent of embryos at that temperature attained stage 20.

Symbols: Solid symbols, normal development; open circle, less than 50 per cent survival.

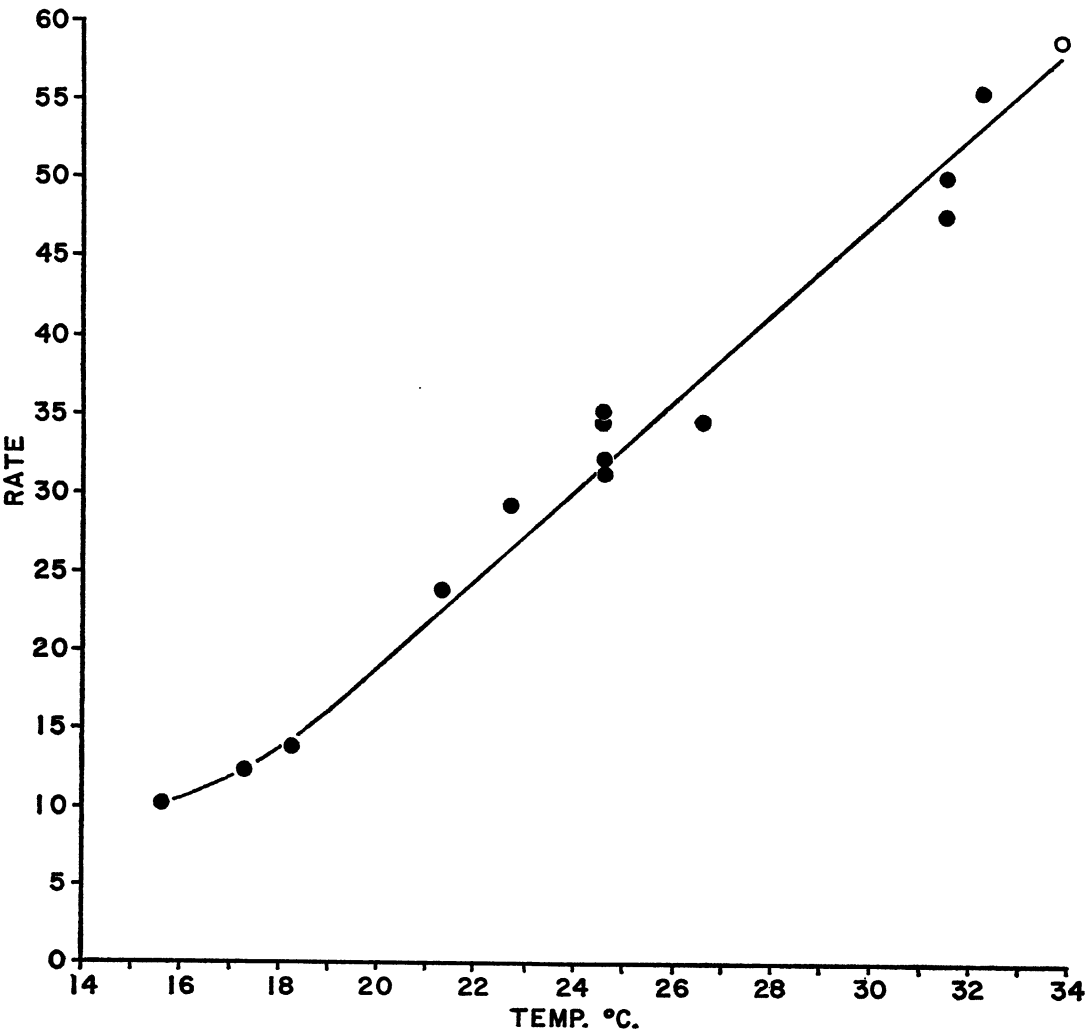


FIG. 17. Rate of development of embryos of *Scaphiopus hammondii* at constant temperatures, expressed as 1000 divided by time in hours to stage 20 ($1/T \times 1000$).

Symbols: Solid symbols, normal development; open circle, less than 50 per cent survival.

RATE OF DEVELOPMENT

The minimum temperature for normal development was not closely approached in the experiments, so the slowest rate cannot be determined. At the lowest temperature for which I have data, 15.6° C., development took 95 to 99 hours. The shortest period observed was 17 hours at 33.8° C. This temperature is evidently about 1° above the maximum for normal development, but the time-temperature curve (fig. 16) is so nearly asymptotic in this region that the maximum rate of development would be little if any less at a slightly lower temperature. At 32.2° C. development took only 18 hours.

Hatching takes place late in stage 19 or in stage 20.

COMPARISON WITH PREVIOUSLY PUBLISHED WORK

Brown (1967) provided an excellent account of the high temperature tolerance of the embryos of *Scaphiopus hammondii* from the Portal area of Arizona. His determination of the upper limit of tolerance under constant temperature conditions is the same that I found, 32.5° C. More important, he determined the extent to which tolerance increases as the embryo grows. He did so by exposing embryos at various stages to higher temperatures for different lengths of time. (My cruder experiments cited herein involved merely placing the embryos at higher temperatures and leaving them there until they either died or developed normally to stage 20.)

Brown found that embryos in early cleavage stages (stages 3–5) could stand three hours at 36.5° C. without dying, but could not tolerate 37.0° C. Embryos in late cleavage (stages 7–9) could tolerate one hour at 37.0° C., but 70 per cent died in two hours. In my experiments no embryos in stages 3 through 7 survived at 35.2° C., although those exposed at stage 7 survived to stage 16. It must be emphasized, however, that these embryos were held at the high temperature rather than being removed to a lower temperature after one to three hours. The difference in our results undoubtedly is due to this factor.

ENVIRONMENTAL CORRELATIONS

TEMPERATURE TOLERANCES IN RELATION TO ENVIRONMENTAL TEMPERATURES

THE SIGNIFICANCE of the temperature tolerances of embryos (see fig. 18 and table 10) must be considered in relation to the temperatures to which eggs are exposed. Unfortunately, reliable and precise information of the sort needed is scarce. References to temperatures at which frogs breed are common enough in the literature, but often only air temperatures are given. Even references to water temperature may be of limited value unless the temperatures are associated specifically with the eggs. A string of *Bufo* eggs a yard long may stretch along a considerable temperature gradient, for example, and gradients may exist even within masses of ova.

An additional complication may be introduced through the heating of an egg mass by solar radiation. A compact mass of dark-colored eggs laid in still water may absorb enough heat to raise its temperature well above that of the surrounding water. An example is shown in figure 19. In this instance, I placed an egg mass of *Rana pipiens* measuring approximately 90 mm. in length, 60 mm. in width, and 35 mm. in depth in a trough of water approximately 100 mm. deep, and at intervals recorded the temperatures within the egg mass and of the surrounding water. By 8:30 A.M., when the first temperature was taken on that particular day, the temperature of the mass was already 1.6° C. higher than that of the surrounding water, and the differential increased to 2.4° C. during the next three hours. Later, clouds interfered with insolation, and temperatures dropped and then rose slightly in intermittent sunlight. The last records taken at 5:20 P.M. after direct sunlight ceased striking the trough show a differential of only 0.4° C., and

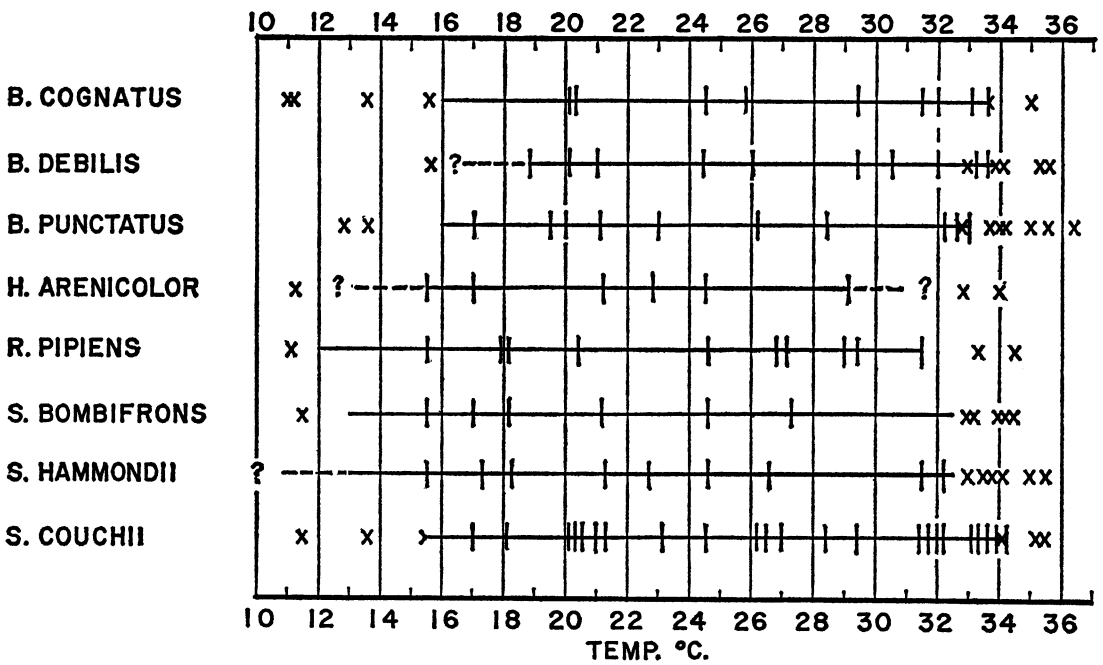


FIG. 18. Ranges of temperature tolerance of early embryos. Horizontal lines indicate estimated ranges of tolerance. Each vertical bar represents one or more experiments with normal development at temperature indicated; X indicates an experiment in which fewer than 50 per cent of embryos survived, presumably because of unfavorable temperature; half-X indicates marginal survival.

TABLE 10

TEMPERATURE RANGE FOR NORMAL EMBRYONIC DEVELOPMENT IN EIGHT FROGS OF THE CHIRICAHUA REGION

Species	Range in Degrees Centigrade	Span in Degrees Centigrade
Bufo cognatus	16.0° to 33.5°	17.5°
Bufo debilis	>15.6°, <18.2° to 33.8°	<18.0°
Bufo punctatus	16.0° to 33.0°	17.0°
Hyla arenicolor	>11.3°, <15.5° to >29.1°, <32.9°	—
Rana pipiens	12.0° to 31.5°	19.5°
Scaphiopus bombifrons	13.0° to 31.5°	19.5°
Scaphiopus couchii	15.5° to 34.0°	18.5°
Scaphiopus hammondii	<15.6° to 32.5°	>17.0°

indicate that the egg mass lost heat rapidly to the water as soon as the input of radiant energy was reduced.

Such differential heating of the egg mass is effective only in still water. A *Rana pipiens* egg mass of similar size placed in the trough in slowly running water at about 24° C. attained a temperature only 0.2° higher than that of the water after several hours of sunlight.

The recent work of Guyétant (1966) provides a welcome exception to the imprecision of many workers in the recording of temperatures associated with embryonic development under natural conditions. He made continuous recordings of temperatures within egg masses of *Rana temporaria* and noted the differential heating (with respect to the surrounding water) of egg masses exposed to the sun. He considered the absorption of heat to be a function of the jelly membranes rather than of the dark-colored ovum or embryo itself, as I think may be the case. Herreid and Kinney (1967) presented similar data for *Rana sylvatica* in Alaska.

The following discussion is arranged according to species and draws both from my own field experience and from the literature. My own data add considerably less information than might be wished for, chiefly because the period during which most critical data might have been gathered in the field often coincided with the busiest in the laboratory. The best temperature data are those associated with ovipositing frogs or with eggs in the earliest stages of development, although water temperatures at which frogs were found in amplexus are almost as good. Lower on the scale of reliability are water temperatures associated with frogs giving mating calls. Frogs probably call over a wider range of temperature than that through which oviposition is initiated.

BUFO COGNATUS

I did not find eggs of *cognatus*, but the water temperatures at which male toads are found calling give some concept of the temperatures to which newly laid eggs may be exposed. I recorded calling at 20.2°, 20.5°, 21.0° (twice), and 22.5° C. Stebbins (1951, p. 252) reported *cognatus* in south-central Arizona calling at body temperatures of 22.0 and 22.2° C., McAlister ("1958" [1959]) found *cognatus* calling in New Mexico in water at 18° C., and Ballinger and McKinney (1966, p. 24) stated: "Water temperature records of breeding choruses range from 15° to 28°."

The only reasonably precise information I have found on the temperatures at which oviposition occurs was given by Bragg (1937, p. 275), who found in Oklahoma toads laying eggs in a pool with a water temperature of 26.0° C. In a later paper, Bragg (1940) presented observations on the relationship of breeding to temperature and concluded that the air temperature critical for breeding (if enough rain has fallen) is 12° C. Unfortunately, Bragg offered few data on actual water temperatures and gave none precisely associated with ovipositing or even calling toads. Possibly water temperatures associated with the air temperature of 12° C. were somewhat higher, perhaps at or above the 16° C. figure

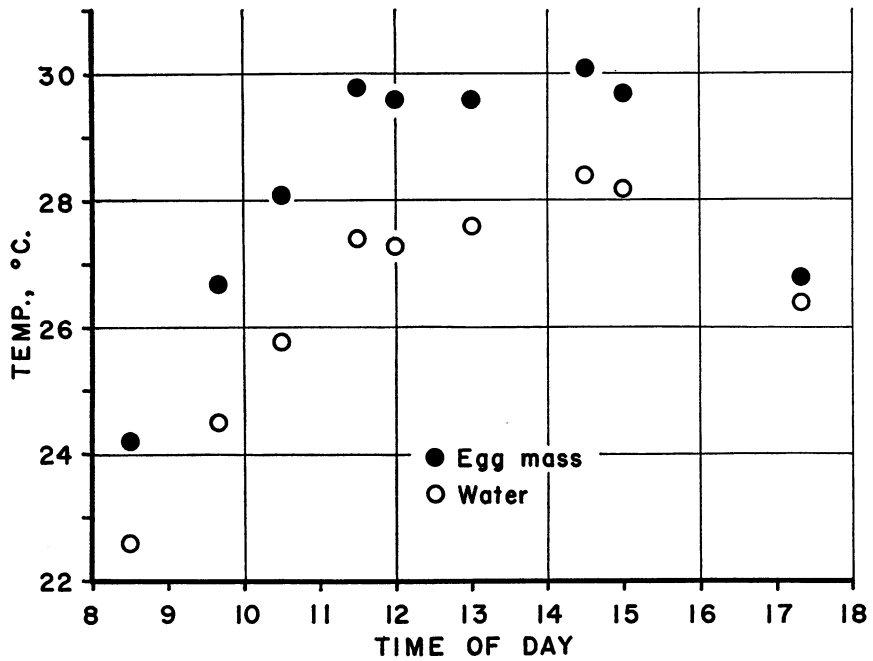


FIG. 19. Relative temperatures of an egg mass of *Rana pipiens* and of surrounding shallow water in sunlight (see text).

estimated as the lower limiting temperature. It seems unlikely that oviposition would be initiated at such a low temperature as 12° C., although, as Bragg personally observed (1940, p. 18), once oviposition is initiated, it may continue even though the temperature falls: "A pair of toads seen laying in a buffalo [bison] wallow at 5:00 P.M., just before the temperature started to fall, finished producing eggs and left the pool despite the lowered temperature."

Bragg (1940) studied *Bufo cognatus* intensively in Oklahoma and concluded that toads of this species breed only after rains when temperatures are not very low. In my study area breeding occurs in the summer months. Presumably the weather is too cool for breeding when it rains in the winter. The spring months typically are without a significant amount of rainfall, but breeding may accompany irrigation of cultivated areas (Brown and Pierce, 1967). It is unlikely that eggs laid in the summer in the region where my study took place are subjected to dangerously low temperatures. Water temperatures in the range of 18° to 22° C. commonly accompany the summer rainstorms, and in summer there are no cold fronts to drive temperatures down once eggs have been laid.

In other parts of the range of *cognatus* conditions may be quite different. In Oklahoma, for example, breeding may take place as early as late March (Bragg, 1940) when temperatures are in the lowest part of the range of tolerance and when a change in the weather may produce critically low temperatures. Presumably a difference such as that between the climates of my study area and of Oklahoma underlies the development of geographic variation in temperature tolerance. In Oklahoma there might well be selection for tolerance of lower temperatures, whereas in my study area the embryos are far less often challenged by low temperatures.

The high temperatures to which embryos of *cognatus* may be exposed are indicated by Bragg's (1940, p. 21) statement: ". . . embryos and larvae develop normally in pools which reach a temperature during the day of 37° C. This is not necessarily the upper limit of tolerance but only the highest at which observations have been made." In the absence of information on the developmental stage of the embryos in question, one should not con-

clude that embryos of the Oklahoma population are more tolerant of high temperature than those in my study area for which I estimate an upper limit of 33.5° C. in the earliest stages.

Bufo debilis

My data for this species are quite limited, as I found no eggs and recorded few temperatures where males were found calling. Water temperatures recorded were 19.4° and 21.0° C. (twice). Stebbins (1951, p. 261) found a male calling beside a rain pool with a water temperature of 22.0° C.

Bufo punctatus

On two occasions I found pairs of these toads in amplexus in water with a temperature of 21.5° C. and twice found males calling in water at 21.0° C. The site where I found the toads in amplexus is a deep, concrete-lined pond that probably never is very warm, but other breeding sites expose the embryos to higher temperatures. For example, I found tadpoles of *punctatus* in shallow potholes, the largest with a maximum depth of 4 inches, in a granite outcrop in the Peloncillo Mountains (pl. 1, fig. 1). The maximum water temperature at the time was 30.9° C. but might conceivably go much higher. More pertinent to the matter of embryonic tolerance is the report by Stebbins (1951, pp. 282–283), who found eggs in a shallow pool of which the temperature of the water at a depth of 1 inch was 32.6° C. Some eggs were on the bottom; others were floating at the surface.

Turner (1959) studied *Bufo punctatus* in Death Valley, California. There breeding takes place in late winter or early spring (toads in amplexus were first seen on March 11 in one year and April 1 in another) and is not dependent upon rainfall. Turner found toads in water at temperatures ranging from 15° to 30° C., but 56 per cent of his records are in the range from 20.1° to 25° C. He noted that mean daily air temperatures below about 21° C. do not seem to be conducive to breeding.

Additional and more specific information on temperature in relation to breeding was provided by Tevis (1966), who studied a population living in the western part of the Colorado Desert in southern California. He found that the toads were not active at air temperatures below 18° C. and did not commence breeding until the evening air temperature reached 24° C. Tevis observed this correlation of temperature and breeding in two different years. He noted (1966, p. 771): ". . . the peak of spawning [evidently over a period of six days], minimum-maximum temperature of the shallow, quiet water in which eggs were laid was 12 to 30° C. . . . Hatching occurred in about 5 days." Eggs of *B. punctatus* would hatch in five days at a constant temperature of about 16.5° C., judged from an extrapolation of the curve in figure 5. This temperature is close to the minimum for normal development, so, where Tevis found the toads ovipositing, the water averaged decidedly cool for *punctatus*.

Observing that the toads became active at an evening air temperature of 18° C. but did not commence breeding until the evening temperature reached 24° C. even though "Change in stream temperature was negligible," Tevis (1966, p. 768) concluded that "temperature of air was the critical factor for initiation of breeding, not water temperature as is true with most anurans." He also reported (p. 769) that oviposition is restricted to sites "1 to 3 in. deep in the quietest water, either at the edge of a slowly moving segment of stream, or in a backwater or inlet between rocks, or in a cove or bay or bank or sandbar."

It seems likely that, although air temperature may be the factor influencing the toads, there still may be an important correlation with water temperature, namely, with the lower range of fluctuation in the shallow sites of oviposition. It is evident from Tevis' data that, even during the breeding period, the temperatures in these sites are relatively low, and they may drop to dangerously low levels when the air temperature early in the evening is only 18° C., even though this temperature permits activity by adult toads (I have found individuals of *punctatus* in California active at a body temperature as low as 14.8° C.). Postponing breeding until the air reaches 24° C. at night may insure against too low temperatures in breeding sites not strongly affected by the moderating influence of the streamflow.

In view of the findings of Turner (1959)

and Tevis (1966) regarding the relationship of temperature to activity in *Bufo punctatus*, Gehlbach's (1965, pp. 269–270) observation is anomalous. He found a large mixed chorus of *Bufo punctatus* and *Scaphiopus hammondii* in a cattle tank at an elevation of 6400 feet in Valencia County, New Mexico, when the water temperature was 53° F. (11.7° C.) and the air temperature 48° F. (8.9° C.). I found that early embryonic development of *punctatus* could not take place even at 12.8° and 13.6° C. If the toads in the region where Gehlbach made his observation actually breed at such a low temperature, their lower limiting embryonic temperature must be vastly different from that of toads in the Chiricahua region.

Hyla arenicolor

In the Chiricahua Mountains and in the Peloncillo Mountains I found calling males of *H. arenicolor* on several occasions. The range of water temperatures and body temperatures was relatively narrow—21.0° to 25.2° C.

John (1964) studied fish in some of the streams where I observed *H. arenicolor* and presented data on water temperatures that are pertinent to my work. The highest temperature he recorded in flowing water in the summer was 31° C., and the lowest, where springs emerged, was 18° C. Shallow, isolated pools in solid rock showed a greater range of temperature. One pool, 2 3/4 inches deep, had surface water as warm as 36° C. in the daytime while the water in the bottom was 33° C. The daily thermal excursion in this pool was 10° to 15° C., but in other pools equally exposed the maximum temperature a few inches down never exceeded 25° C.

Hyla arenicolor breeds in the Chiricahua Mountains in the spring when the canyon pools still retain water from the melting snow at high elevations and again in summer when (or if) the water in streams and pools is augmented by local storms (Zweifel, 1961). Judged from John's data, dangerously low temperatures are not likely to occur in summer, but temperatures above the upper level of tolerance are present in some sites. In the spring, temperatures are much lower. John (1963) mentioned daily maximum and minimum stream temperatures of 15° and 8° C. in April, but it is not certain that *H. arenicolor* breeds so early in the year. Basing my estimate on the size of larvae collected in June, I inferred that breeding took place in May (Zweifel, 1961, p. 16). Presumably the relatively low minimum temperature tolerance of *arenicolor* (less than 15.5° C. but above 11.3° C.) selectively adapts the embryos to the moderately cool conditions prevailing in mid-spring after the runoff from melting snow has ceased. Eggs laid in the summer are unlikely to meet intolerably high temperatures unless laid in exposed, shallow pools. The only site of oviposition that I observed was at a depth of several inches in a pothole shaded most of the day (pl. 1, fig. 3).

Rana pipiens

In the Chiricahua Mountains this species breeds throughout a large part of the year. Mont A. Cazier (personal communication) found eggs on February 7, 1960, and February 14, 1961. The latter were the first of the year to be laid in a spring-fed swimming pool on the grounds of the Southwestern Research Station. Cazier also recorded eggs found in May, and I noted eggs in June, July, August, and early September. Lack of observation rather than lack of oviposition probably explains the hiatus in March and April.

Water temperatures associated with eggs of *R. pipiens* that I observed in the Chiricahua Mountains ranged from 22.8 to 25.2° C. I noted that after a period of heavy rain in August, when the water temperature in a favorite site of oviposition dropped to 14.5° C., there was a conspicuous lack of recently deposited eggs, although gravid female frogs were present. The correlation with water temperature may be spurious, however, for another factor such as increased water turbidity may have been influential. Breeding early in the year may be limited to spring-fed sites (such as the swimming pool mentioned above) where the water temperature is not too low. Data given by John (1963) concerning stream temperatures in April (see foregoing account of *Hyla arenicolor*) are pertinent in this regard.

On June 24, 1958, 4 miles south-southwest of Alpine, Apache County, Arizona, I found *R. pipiens* in amplexus and eggs freshly laid in water of 13.5° C. This is fairly close to the

estimated minimum temperature for normal development in *pipiens* of the Chiricahua Mountains (12° C.). It would be of interest to determine whether *pipiens* of higher elevations north of the Mogollon Rim (the locality is at an elevation of 8400 feet, 130 miles north of the Chiricahua Mountains) represent a more cold-tolerant "race."

In the Peloncillo Mountains I found an egg mass from which larvae were hatching in the lower half, whereas all embryos had died in the upper half. The possibility that the surface layer of water had become too warm for the embryos immediately suggested itself (the eggs were unshaded), but the possibility that the water level may have dropped enough to expose the upper part of the mass and then had risen again cannot be ruled out. This site was unusually shallow and unshaded. Where I found eggs in the Chiricahua Mountains, they were either deep enough to be protected from the sun or were sheltered by overhanging, heavily vegetated banks. High temperatures would not seem to present a danger to the embryos in these habitats.

Scaphiopus bombifrons

Water temperatures at which I observed calling ranged from 18° to 23° C., and oviposition took place on nights when temperatures of 18.0° and 21.5° C. were recorded. Embryos were exposed to a water temperature of 28.6° C. by 11:00 A.M. the day following oviposition at 18.0° C. and were hatching in water at 33.0° C. by 10:45 A.M. the following day. When the water temperature had been 21.5° C. at oviposition in another instance, it was 29.5° C. by 9:55 A.M. the following day. (See the account of *S. hammondii* for a summary of temperature conditions in temporary pools.)

I have found little useful information in the literature on water temperatures associated with breeding or calling in this species. McAlister ("1958" [1959]) found individuals of *bombifrons* in central New Mexico calling in water at 18° C. Hoyt (1960, p. 199) reported a water temperature of 22.4° C. in a pond in Kansas in which *S. bombifrons* was breeding. Bragg and Smith (1942, p. 43) studied *S. bombifrons* in Oklahoma and, on the basis of the occurrence or absence of breeding following heavy rains under a variety of temperature conditions, "set the [air] temperature below which they do not ordinarily breed as close to 11° C." This is a little below the lower limiting temperature of about 13° C. that I estimated, but slightly higher water temperatures probably would accompany the air temperature cited.

Another observation by Bragg and Smith (*loc. cit.*) pointed up the desirability of being specific about the association of oviposition and water temperature. They found *Scaphiopus bombifrons* "attaching eggs to leaves of Polygonon sp.—water temperature at the surface, 38° C., six to eight inches below, 29° C." Were the toads ovipositing at the higher temperature indicated, which is far above the upper limiting temperature for *bombifrons* (or any other species for which information is available)? Probably the eggs were being laid in cooler water well below the surface.

Scaphiopus bombifrons, with its embryos tolerant of relatively low temperatures, could breed in the spring months, but it is doubtful that rains at this time in my study area are ever sufficient to stimulate breeding. (See remarks in the account of *S. hammondii*).

Scaphiopus couchii

In my study area I found *S. couchii* in amplexus in water at 19.5° and 21.5° C., and at Hermosillo, Sonora, I encountered a large chorus in a rain pool with a water temperature of 24.6° C. Near Guaymas, Sonora, I found eggs hatching in water at a temperature of 26.6° C.

Wasserman (1957) compared sympatric populations of *Scaphiopus couchii* and *S. holbrookii hurterii* in the vicinity of Austin, Texas, with respect to air temperatures at the sites of breeding congresses. The two lowest temperatures he noted for *couchii* were approximately 17° and 18° C. In seven other instances the temperature was 20° C. or higher (Wasserman, 1957, fig. 2). Arnold (1943) found individuals of *couchii* in amplexus in southern Arizona where the air and moist soil temperatures were about 22° to 23° C.; probably the water was about the same temperature.

Observations in my study area merit comparison with those of Wasserman. On July 2, 1958, a heavy thunderstorm (almost cer-

tainly the first of the season) filled a temporary pool 6 miles east-southeast of Portal (pl. 2, figs. 1, 2); the amount of rain that fell is not on record. In the evening, when the water temperature was 21.5° C., a strong chorus of *Scaphiopus couchii* and *S. bombifrons* developed in the pond, and both species bred. A few individuals of *S. hammondii* also were present, but I am not certain that breeding took place. On July 8, 1960, the first storm of the season to strike this particular area deposited 1.49 inches of rain (the rain gauge was within a few feet of the pond) between 8:00 P.M. and 10:30 P.M. and filled the pond. At 10:30 P.M., when the rain had almost stopped, the water temperature was 18.0° C., and *S. bombifrons* and *S. hammondii* were calling in large numbers. At least one and probably both species bred, but *S. couchii* was conspicuously absent.

Temperature is the only obvious variable that correlates with the activity of *S. couchii*. In both instances the storm was the first of the year to fill the pool and was of sufficient intensity to stimulate activity by *S. bombifrons* and *S. hammondii*. Wasserman's data indicated that an air temperature between 17° to 18° C. is marginal for breeding, and my observations support this inference. It should be recalled that the lower limiting temperature for embryonic development appears to be the same for frogs in Wasserman's study area as it is in mine. (See the account of *S. hammondii* that follows for a summary of temperature conditions in the temporary pools in which *S. couchii* breeds.)

Scaphiopus hammondii

I found *S. hammondii* calling in my study area at water temperatures of from 18° to 23° C. and found many pairs in amplexus in a pool with water temperatures of from 20.8° to 21.4° C. Brown (1967, p. 366) reported breeding initiated at water temperatures of from 18° to 24° C. on two occasions in the same region. Most other records for this widespread species fall within the same range of temperatures. Calls were recorded in Coahuila, Mexico, at 18° C.; in Oaxaca, Mexico, at 18° C.; and in Jalisco, Mexico, at 20.5° to 21.5° C. (library of tape recordings in the American Museum of Natural History). The lowest temperature on record was reported by Gehlbach (1965, p. 269), who found these frogs ovipositing in water at 53° F. (11.7° C.) in northeastern New Mexico.

In view of the tolerance embryos of *hammondii* have for low temperatures, it is unlikely that embryos in my study area developing in the summer are ever exposed to dangerously low temperatures. This tolerance for low temperatures would permit breeding in the spring, but this season characteristically is quite dry. In 33 years of record the total monthly rainfall at Portal exceeded 1 inch only six times in April and three times in May (Green and Sellers, 1964). Breeding in the spring months when low water temperatures might be encountered must occur rarely, if at all.

Temperature Conditions in Temporary Rain Pools

Inasmuch as the three species of *Scaphiopus* confine their breeding to temporary pools or to ecologically similar cattle tanks, it is useful to gather in one place my observations on conditions in this habitat.

The pools receive their water directly from rainfall and local runoff, and temperatures during summer rains evidently do not vary greatly. I recorded water temperatures of from 18° to 23° C. while species of *Scaphiopus* were breeding during or shortly after rains, and the observations by Brown (1967, p. 367) in the same region extend this range slightly to 24° C. Undoubtedly, lower temperatures occur, but I doubt that the limit I observed is exceeded by more than one or two degrees. On one occasion we encountered an unusually violent storm, with rain and hail, but the water temperatures where we found individuals of *Scaphiopus* breeding shortly thereafter were no lower than 19.2 to 19.8° C. The cooling effect of the storms is such that water temperatures when the frogs are breeding are not likely to exceed by much the upper limit of 24° C. cited.

Although water temperatures typically are moderate during and shortly after rains when the eggs are laid, they do not remain so the following day if, as is usual, the clouds have dispersed and the pools are exposed to full sunlight. So far as embryonic development of *Scaphiopus* is concerned, only the first 48 hours, at most, are of consequence; even if

the temperature remained as low as 20° C., embryos of all of the three species would hatch in about this period of time.

Intermittent observations that I made at the same pool following the breeding by *Scaphiopus* in two different years suggest that the following may be a fairly typical temperature cycle: At sunrise the water is at approximately the temperature it was when the rain fell, about 18° to 23° C., with little or no difference between shallow and deep (about 1 foot in the pond studied) parts of the pool. The shallow marginal waters heat up rapidly as the sun rises; by 11:00 A.M. the temperature may reach 30° C., and it may rise above 35° C. by early afternoon. In late afternoon the water cools rapidly, and by 8:00 P.M. its temperature drops to as low as 20° C. The increase in temperature in deep water lags behind that of the shallows. On the morning following the rain, although all the water was relatively cool, the difference between shallow and deep water was slight. For example, at 9:10 A.M. the shallow temperature was 23.7°, whereas on the pond bottom at a depth of about 1 foot the temperature was 22.2° C. By 11:00 A.M. the temperatures were, respectively, 30.7° and 28.2° C. Conditions are likely to be warmer at the same hours on subsequent days if no additional rain falls. At 10:45 A.M. on the day following the one mentioned, the shallow temperature was 35.2° C., and the deep temperature was 30.7° C. The frogs do not place their eggs in the shallowest (featheredge) part of the pond, so they avoid the highest temperatures. On this particular occasion when the shallows were at 35.2° C. eggs slightly farther out in the pond were hatching at 33.0° C.

The highest temperature I noted in this pond was 37.1° C. at 3:30 P.M., but this maximum was reached only after several days without rain when the depth of water was greatly reduced. The air temperature at the time was 40.3° C. The lowest temperature I recorded was 17.5° C. at 10:15 P.M. when the water was almost gone and the air was at 30.0° C. The effect of evaporative cooling on shallow pools in this arid habitat is thus quite striking. Even under intense insolation and high air temperatures the water temperatures do not rise so high as might be anticipated; late one afternoon when the air had reached 42.8° C. (thermometer bulb shaded), the shallow water was still only 33.0° C.

Data highly similar to mine on water temperatures associated with breeding and development of *Scaphiopus hammondii* were gathered by Brown (1967, p. 366) in the same region as mine. The only slight difference in our data is that he recorded a maximum of 39.0° C. for embryos in stage 15 (rotation), almost 2° C. higher than the highest I noted.

In summary, temperatures in temporary rain pools are moderate, about 18° to 24° C. during and immediately after rains when the individuals of *Scaphiopus* breed. Neither the upper nor lower temperatures limiting for embryonic growth for any of the three species are closely approached at this time. Temperatures verging on those dangerous for early embryos of *S. bombifrons* and *S. hammondii*, less so for *S. couchii*, may be reached by early afternoon, within perhaps 12 to 14 hours, or less, of the time when oviposition takes place. By this time, however, the embryos will have developed through the state of gastrulation and will thus have passed the time during which they are most sensitive to damage by high temperatures.

RATE OF DEVELOPMENT IN RELATION TO HABITAT

Rates of embryonic development in the eight species studied are summarized in figures 20 and 21. The rates seen here virtually run the gamut of those known for species with typical embryonic development (that is, excluding species with "direct development" or other modification): *Scaphiopus* shows perhaps one of the highest rates attained by anurans, and *Rana* is among the slowest.

The curves (figs. 20 and 21) assort into three groups: one contains the three species of *Scaphiopus* with their rapid rates of development; the second includes those of *Hyla* and the three species of *Bufo;* and the third includes only *Rana pipiens* with its extremely low rate of development. Given

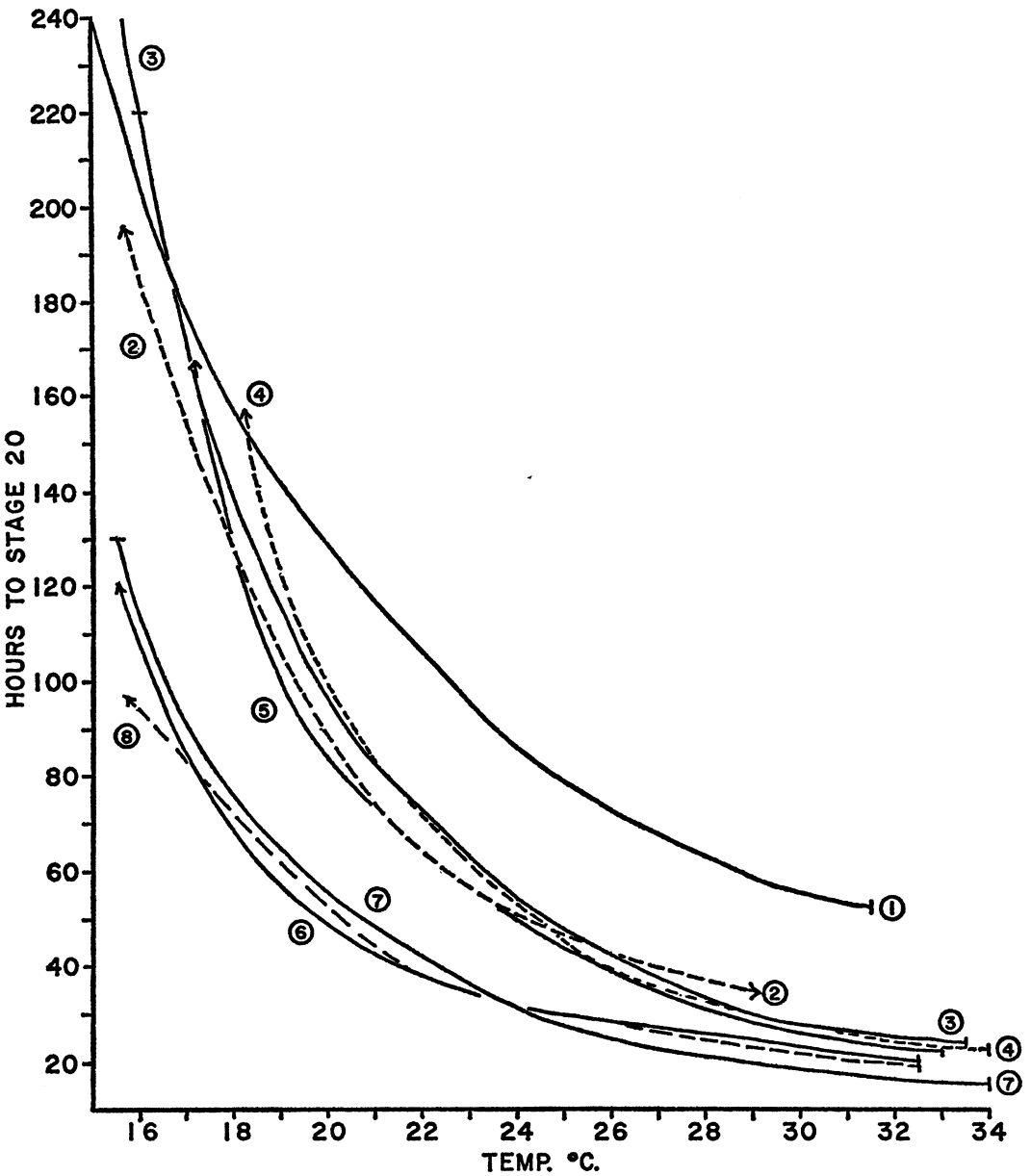


FIG. 20. Time required to reach stage 20 by embryos of several species of frogs incubated at constant temperatures. 1. *Rana pipiens*. 2. *Hyla arenicolor*. 3. *Bufo cognatus*. 4. *B. debilis*. 5. *B. punctatus*. 6. *Scaphiopus bombifrons*. 7. *S. couchii*. 8. *S. hammondii*.

these vastly different rates among species living in the same region and to some extent breeding in the same habitats, the question of the adaptive significance of the differences arises. Do the different rates confer adaptive advantages in particular habitats? Does having a particular rate of development at a given temperature restrict oviposition to certain sites?

There is a clear correlation between the rate of development and the length of time the breeding site may be expected to retain water. At one extreme, *Scaphiopus* has extremely rapid rates of both embryonic and

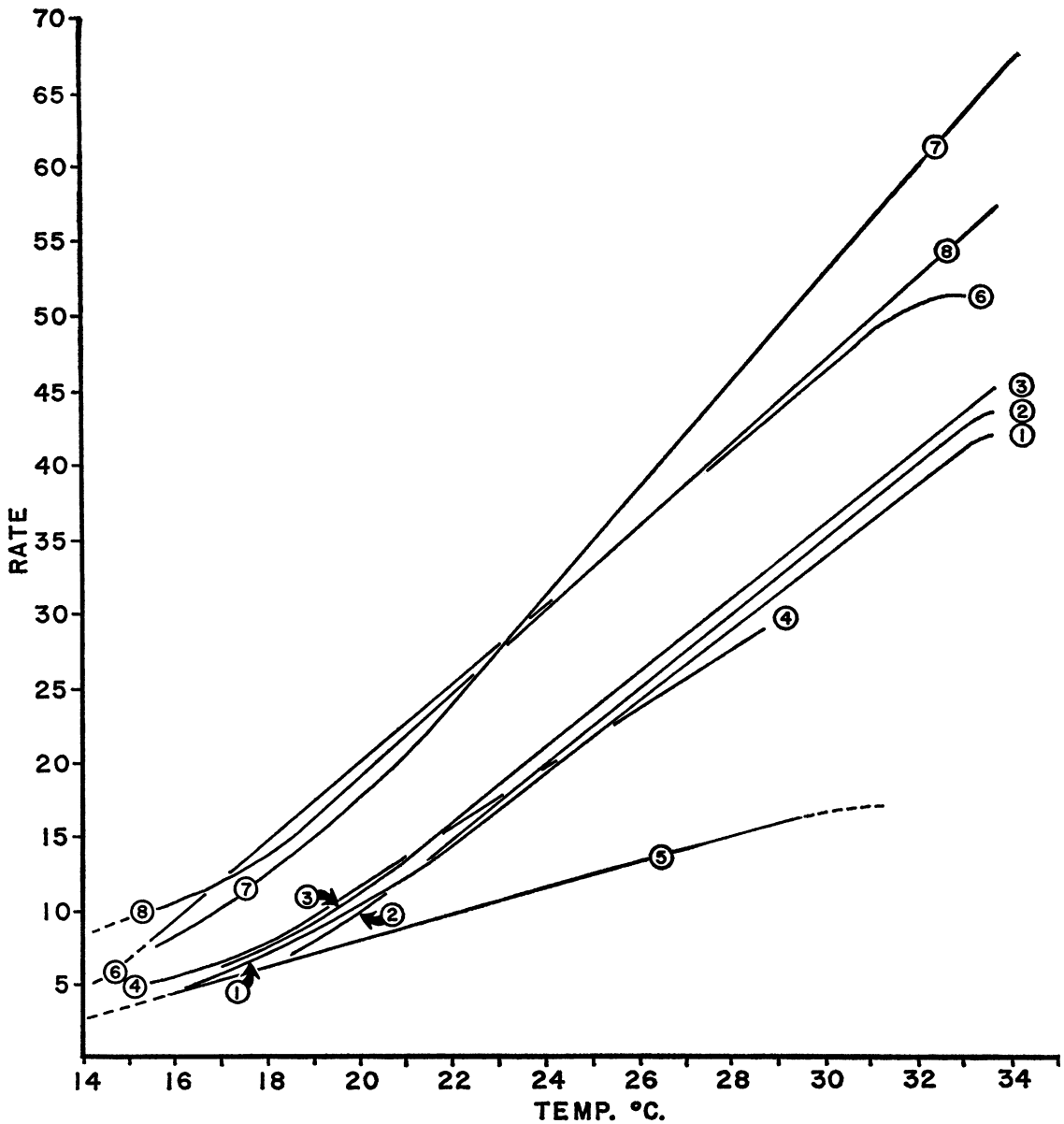


FIG. 21. Rate of development of embryos of several species of frogs incubated at constant temperatures, expressed as 1000 divided by time in hours to stage 20 ($1/T \times 1000$). 1. *Bufo cognatus*. 2. *B. debilis*. 3. *B. punctatus*. 4. *Hyla arenicolor*. 5. *Rana pipiens*. 6. *Scaphiopus bombifrons*. 7. *S. couchii*. 8. *S. hammondii*.

larval development and breeds in temporary rain pools from which more often than not the water is gone even before the larvae are able to metamorphose. *Rana pipiens* lies at the other extreme; it has the slowest rate of embryonic development and breeds (in my study area, at least) only where the water supply is permanent.

The three species of *Bufo* together with *Hyla arenicolor* form the intermediate group with respect to rate of development and, appropriately, often breed in sites with capac-

ities for water retention that are intermediate between the extremes cited above. *Hyla arenicolor* sometimes utilizes the same sites as *Rana pipiens*, but also breeds in intermittent watercourses where the pools are replenished only by variable and unreliable local rainfall. *Bufo punctatus* breeds in situations quite similar to those used by *Hyla*. The other two species of *Bufo* are sometimes found with the individuals of *Scaphiopus* in temporary pools but are more likely to breed in ditches or irrigated fields where the water supply lasts longer.

The breeding sites of *Hyla* and *Bufo punctatus* on the one hand and those of the three species of *Scaphiopus* on the other are similar in that the water may result from only one local storm. The pools of *Scaphiopus* form in the fine soils of valleys where both seepage through the substratum and evaporation act quickly to reduce the volume of water, whereas *Hyla* and *Bufo* often breed in potholes scoured in rock from which there is little or no loss through seepage. A pothole filled by one rain may hold water for many weeks, but a typical desert rain pool may not retain water for a sufficient period of time to permit the *Scaphiopus* tadpoles to metamorphose unless additional storms replenish the supply.

A rapid rate of development clearly is an adaptive advantage in situations where the water supply may quickly dwindle or even fail, but it is not evident why relatively slower rates of development are associated with breeding sites that have permanent water. One might assume that a rapid rate of development would be advantageous in all situations. Perhaps a rapid rate is attained or maintained only when there is particularly rigorous selection for it because of the transient nature of the water supply or other factors (see below).

In general, species with rapid rates of embryonic development have rapid rates of larval development, and these in turn correlate with the permanency of the water supply of the breeding site. An additional important consideration with respect to rate of development is discussed in the following section.

MODES OF ADAPTATION TO TEMPERATURE

A FROG EMBRYO EXHIBITS two inherent adaptations to temperature: (1) a tolerance, in early embryonic stages to temperature over a range that is species-constant, or at least is relatively constant within a widespread population; and (2) an increase in the range of this tolerance as embryonic development proceeds. The form of the egg mass and size and color of the ovum may be influenced by or may influence temperature, and also intimately involved in adaptation to temperature is the important extrinsic factor of parental selection of time and place of breeding.

RANGE OF TOLERANCE

EARLY RANGE OF TOLERANCE

The range of temperature within which an early embryo can develop is subject to limitations that no species appears to transgress. Embryos of a few species can survive, although not necessarily develop, close to the freezing point, but these are exceptional (the majority of frogs are tropical). Available evidence indicates that the upper limiting temperature for early embryos is not likely to exceed 35° C. by much in any species. Volpe found this limit in three species of *Bufo*; and Moore, in one population of *Rana pipiens*. The highest I found in desert anurans was 34° C.

Exceptions may exist. Balinsky (1957) reported "normal development" of three South African species at 36° C. and cited "lethal" temperatures of 39° and 40° C. for these species. It is not certain, however, that his experimental method, particularly with reference to the exposure of the embryos at or before first cleavage, was directly comparable to that under which the tolerances for American species were determined.

The total span of about 4° to 35° C. is not seen in any one species, and spans in the neighborhood of 20° C. are fairly typical (see tables 6, 10). The reasons are not clear. Is it physiologically impossible for an early embryo to be adapted to both high and low temperatures simultaneously? I suspect that the reason may be as much ecological and evolutionary as physiological. There are few situations in which a breeding population would be subjected to the selective action of both high and low temperatures while embryos were in an early stage of development, so there would be little premium on ability to develop over a wide range of temperature.

ONTOGENETIC CHANGE IN TOLERANCE

Perhaps more important than the span of temperature tolerated by an early embryo is the broadening of the span that takes place as the embryo passes through cleavage stages and continues into larval life. Although it has long been known that anuran embryos become increasingly tolerant of high and low temperatures as development proceeds (Atlas, 1935; Schechtman and Olson, 1941; Moore, 1942b, pp. 380–381, footnote), the ecological significance of this change in tolerance has not been widely appreciated. Indeed, some authors have not differentiated between temperature tolerances of early embryos and those of larvae. The recent paper by Brown (1967) is the only one of which I am aware that dealt extensively with the tolerances of embryos through various developmental stages and examined the data from an ecological point of view. Herreid and Kinney (1967) presented some similar information on *Rana sylvatica*.

Brown (1967, pp. 368–369) stated: "The possession of a very rapid developmental rate by the spadefoot [*Scaphiopus hammondii*] has the effect of removing the more heat-sensitive stages from exposure to high environmental temperatures. In this manner the rapid developmental rate may compensate for a lower upper limiting temperature." It has been customary to consider that the rapid developmental rate of *Scaphiopus* embryos is an adaptation to the ephemeral nature of the breeding ponds, but it is evident from Brown's data (and to a lesser extent from mine) that the increase in temperature tolerance that accompanies development may be much more important to the embryo exposed to typical rain-pool conditions than

the few hours gained in total time of development. Adaptation to water of high temperature may well involve selection for an increase in rate, as well as for (or in place of) increased temperature tolerance in the early embryo.

With these considerations in mind, we may picture what might occur if the slowest- and fastest-developing species were to breed simultaneously in a desert rain pool under the typical conditions outlined earlier. Eggs of *S. couchii* laid at a temperature of 20° to 21° C. would be in late cleavage (stage 9) or even commencing gastrulation (stage 10) by the following morning about eight hours later, whereas those of *R. pipiens* would only be in early mid-cleavage (stage 8E). The embryos of *couchii* would thus pass the critical stage of gastrulation before being exposed to the high temperatures of the afternoon, while those of *pipiens* might well be subjected to a lethally high temperature even before gastrulating.

Because embryos of all species studied undergo an increase in tolerance to high temperature as they develop, it is pertinent to inquire whether species differ in the rate at which their tolerances increase. It would not, for example, be astonishing to find that the upper limit of tolerance of *Scaphiopus hammondii* (early upper limit, 32.5° C.) increased more rapidly relative to stage of development than that of *Rana pipiens*, which initially has a similar upper limiting temperature (31.5° C.). Unfortunately, my experimental data (see species accounts) are too incomplete for adequate interspecific comparisons to be made.

No embryos of *Rana pipiens* raised at a moderate temperature and then transferred at stage 8E to water that fluctuated between 2.1° and 2.9° C. above the estimated upper limiting temperature survived to hatch, although others held at only 1° above the limiting temperature developed normally. Embryos of *Bufo punctatus* and *Scaphiopus bombifrons* treated similarly developed normally at temperatures about 1.8° C. and 1.9° C., respectively, above their limiting temperatures. That embryos of *S. bombifrons* survived at +1.9° C., whereas those of *R. pipiens* failed at +2.1° C., suggests the possibility of a more rapid attainment of heat tolerance in *bombifrons*, but the data are too few for a meaningful conclusion. Embryos of *punctatus* survived 16 hours at 3.4° C. above the normal limit before successfully finishing their development at temperatures that were lower but still above normal. This situation is perhaps more significant in that it simulates an exaggerated natural one in which the temperature increases and remains high for several hours before returning to a more moderate level.

The single experiment with *Scaphiopus hammondii* indicates that the embryos attain greater tolerance for high temperature rather rapidly and relatively early in development. Embryos placed at 2.7° C. above the upper limiting temperature while in stage 6 died in stage 9, prior to the commencement of gastrulation. Embryos of the same clutch not exposed to the higher temperature until stage 7 gastrulated and survived until stage 16.

Scaphiopus couchii among the species studied is clearly the best adapted to high temperatures. It not only has the highest upper limiting temperature (34° C.), but also has an extremely rapid rate of development that assures the rapid attainment of even higher limits of tolerance. The species most broadly adapted to temperature is *Scaphiopus hammondii*. The rather low lower limiting temperature permits breeding under conditions colder than can be tolerated by many other anurans with which it lives, but its rapid developmental rate permits breeding where extremely warm conditions may prevail within a few hours of oviposition.

OTHER ADAPTATIONS IN EMBRYONIC STAGES

Although my work was concerned principally with temperature tolerances and developmental rates, it is appropriate to discuss briefly four other aspects of adaptation in the egg and embryonic stages: the color and the size of the ovum, the form of the egg mass, and the stage at which the embryos hatch.

Color of Ovum

The color of the ovum is determined by the quantity and distribution of melanin and, hence, by the extent to which it masks the underlying yellowish white yolk. Typically, melanin is concentrated in the animal hemisphere, although in especially dark eggs the coverage extends well over the vegetal part of the ovum.

The darkest eggs are those of the toads *Bufo punctatus* and *Bufo cognatus* and of the spadefoot *Scaphiopus couchii*. In these the upper hemisphere (in some cases a little more, in others a little less) is dark brown, virtually black in some instances. The ova of *Rana pipiens* are similar. The ova of *Bufo debilis* are considerably paler than those of the other two species of *Bufo* and tend to be slightly darker in the equatorial region, giving them the appearance of a monk's tonsure. The remaining two species of *Scaphiopus* (*bombifrons* and *hammondii*) have eggs that are distinctly paler than those of *S. couchii*, and the dense melanin does not extend so far into the equatorial region. I have no information on the ova of *Hyla arenicolor*.

Dark coloring in the ovum may promote absorption of radiant heat energy. If so, it may explain the presence of such coloration in *Rana pipiens* breeding in relatively cool waters and its reduction in forms breeding in lowland waters exposed to intense insolation. In this respect, however, the evidence from *Scaphiopus* and *Bufo* is somewhat contradictory. All three species of *Scaphiopus* breed synchronously in the same ponds, and *couchii* has noticeably darker eggs than the other two. *Bufo debilis* and *B. cognatus* evidently breed sympatrically but have eggs of distinctly different degrees of darkness.

Probably the density of pigment in the ovum is influenced by more than one selective factor. Heat absorption may be one, and shielding from ultraviolet radiation may be another. Anuran eggs laid in situations sheltered from light (e.g., those of *Ascaphus truei*) typically have little or no dark pigment. The paleness of the eggs of *Scaphiopus bombifrons* and *S. hammondii* may be related to the normally turbid condition of the breeding pools, with consequent reduction of light intensity. The eggs, however are frequently attached to submerged vegetation rather close to the surface of the water, where interruption of light by turbidity would be minimal. A historical factor may also be involved. For example, *Bufo debilis* and *B. cognatus* may have evolved under conditions in which different selective forces acted on the melanization of the ova, and in their present areas of sympatry selective forces are not strong enough to modify the established trends.

Size of Ovum

Moore (1942a) noted that northern species of *Rana* (the embryos of which are adapted to lower temperatures) differ from southern species in having larger eggs, and he found a similar trend within *Rana pipiens* (Moore, 1949b). Thus it is of interest to compare the sizes of ova of the species in my study area. (All measurements given are of uncleaved ova preserved in formalin unless specified otherwise and refer only to the vitellus itself without its surrounding jelly membranes.)

The eggs of *Bufo* are the smallest. A sample of 25 ova of *B. debilis* averaged 0.98 mm., and another sample of 32 (measured before preservation) averaged 1.15 mm. Two samples (25 each) of *B. punctatus* averaged 1.18 and 1.31 mm., and the average size of 25 ova of *B. cognatus* was 1.13 mm. The averages I determined for these two species are similar to those reported for *cognatus* by Bragg (1937, p. 281: mean, 1.18 mm.) and for *punctatus* by Livezey and Wright (1947, p. 190: range, 1.0–1.3 mm.).

The eggs of the spadefoots are distinctly larger than those of *Bufo*. Two samples of *Scaphiopus couchii* (25 each) averaged 1.34 and 1.44 mm. A sample of 25 ova of *S. bombifrons* averaged 1.58 mm., in close agreement with the mean of 1.5 mm. given by Trowbridge (1941, p. 509). Two samples of *S. hammondii* (25 each) measured before preservation gave rather different means: 1.60 mm. (1.56–1.64) and 1.16 (1.09–1.25). The total range of both of my samples is almost within the range for the species given by Livezey and Wright (1947, p. 192: 1.0–1.62 mm.).

Unfortunately, I have no information on the sizes of the ova of either *Rana pipiens* or *Hyla arenicolor* in the Chiricahua region. Livezey and Wright (1947, p. 189) gave the mean diameter of the eggs of *arenicolor* as

2.07 mm., but *H. arenicolor* was not distinguished from *H. californiae* at the time they wrote, so it is uncertain whether their data apply to one or both species. Measurements of the eggs of "*Hyla arenicolor*" (=*H. californiae*) given by Storer (1925, p. 210) are closely similar to those given by Livezey and Wright.

There is little in the data presented here to suggest a correlation of the sort Moore found in *Rana*. The ova of *Scaphiopus couchii* probably average slightly smaller than those of *S. bombifrons* and *S. hammondii*, and *couchii* is characteristic of warmer, although not necessarily more southern, regions. Not even this slight distributional correlation is evident in *Bufo*; the slightly smaller size of *debilis* eggs may merely reflect the smaller body size of that species.

Moore (1942a) noted that a slight difference in egg diameter implies a sizable difference in the amount of stored yolk available to the embryo and newly hatched tadpole. The "northern" species thus endow the tadpoles with a more abundant food supply that enables the attainment of a larger size before an external source of food is needed. Conceivably, such a source of food might be of more importance to a larva of *Scaphiopus* hatched in a newly formed and at first somewhat oligotrophic pond than to a tadpole of *Bufo* in a more permanent pool.

Egg size is inextricably bound up with egg number. Some evolutionary lines (*Bufo* is a good example) rely for their continued existence on producing extremely large numbers of offspring, which places a limit on the amount of yolk that can be provided each ovum. Other lines have evolved in which fewer but better-endowed eggs are produced (*Scaphiopus* may tend in this direction). Making comparisons that cut across widely divergent phyletic lines would have very little value.

Form of Egg Mass

Considerable variety is seen in the types of egg mass deposited by anurans of the Chiricahua region. Females of some species of anurans typically deposit their eggs singly, whereas others group the eggs together in clumps of various but characteristic shapes and sizes.

Campbell's (1934, p. 5) account of the eggs of *Hyla arenicolor* observed in the Peña Blanca region of Arizona stated: "[In] large pools they were attached singly to the weeds and brush or to the stones on the bottom; some floated on the surface, probably broken off from their attachments. In the very small potholes in the rocks the eggs were in large clumps." With reference to *Hyla californiae*, Storer (1925, p. 214) suggested that single eggs would be better able to withstand buffeting by a sudden torrent, a frequent occurrence in the stream habitat, than would eggs laid in a clump. This explanation would apply with equal force to *Hyla arenicolor* and possibly also to *Bufo punctatus*. The last species is unusual among species of *Bufo* in that the eggs are not deposited in long strings, but are laid singly in short files, or in a loose scattered mass on the bottom (Livezey and Wright, 1947, p. 204). When breeding in streams, *Bufo punctatus* may typically select shallow, quiet marginal situations (Tevis, 1966, p. 769). But even there the danger of disruption by a sudden increase in current flow exists.

Bufo cognatus is typical of the genus in that the eggs are produced in long strings. This manner of deposition gives excellent exposure of the embryos and presumably is well adapted to breeding in warm, still water. It also functions well in relatively cool situations, such as those utilized by *Bufo americanus*.

The eggs of *Bufo debilis* are inadequately known. Strecker (1926, p. 10) described them as being "in small strings . . . attached to grass and weedstems." My observations on eggs laid in the laboratory confirm only the fact that they are not produced in the long strings typical of most species of *Bufo*.

Egg masses of *Scaphiopus bombifrons*, *S. couchii*, and *S. hammondii* are similar to one another in that they consist of relatively small numbers of eggs, up to about 250. The eggs may be in a string or cylinder almost like that of *Bufo* (*S. couchii*), in strings of eggs not encased in a common envelope of jelly (*S. hammondii*), in cylindrical masses (*S. hammondii*), or in elliptical clusters (*S. bombifrons*) (Livezey and Wright, 1947). A common point is that all masses evidently have a relatively large surface-to-volume ratio,

conceivably of adaptive value in warm-water situations.

Rana pipiens of the Chiricahua Mountains deposits its eggs in a large, globular mass just as do the populations of the northern regions and other northern species studied by Moore (1940).

Moore (1940) noted that northern populations of *Rana* breeding in relatively cool waters (*R. sylvatica*, *R. palustris*, and northern populations of *R. pipiens*) deposit the eggs in a submerged, globular mass, but species breeding under warmer conditions (*R. clamitans* and *R. catesbeiana*) lay the eggs in a thin film at the surface of the water. This difference is interpreted as adaptive with respect to meeting the oxygen needs of the embryos. Cool water can hold more dissolved oxygen, and the embryos in the middle of the globular but porous mass do not suffer. In the low-oxygen tension prevailing in warmer water, a surface-film type of mass provides the maximum exposure for each embryo. An additional factor is that in still water the concentration of dark pigment in the globular mass promotes absorption of heat and, hence, speeds development. Eggs in a surface film have the maximum opportunity for respiratory exchange and, because they are rather dispersed, are not likely to concentrate heat. The small masses or strings of eggs seen in *Bufo* and *Scaphiopus* appear to be well adapted to warm-water conditions in that they allow fairly free exposure of the eggs to the surrounding water.

Considerable variation is reported in the number of eggs per mass and in the shapes of the masses of some species, including the three species of *Scaphiopus* studied here (Livezey and Wright, 1947). It would be of interest to know if the form of an egg mass might vary according to the temperature of the water in which it was deposited. The size and shape of an egg mass must, in most instances, depend on the behavior of the mated pair during oviposition, and this could be influenced by temperature. Smaller, less compact masses may be produced at high temperatures; if so, it could be interpreted as an adaptive response.

Stage at Hatching

The species of anurans with typical aquatic development exhibit considerable variation in the stage of development at which the embryo (or larva) leaves the jelly membrane. Hatching is typically quite early in *Bufo*, in stage 16 or 17 before muscular movement is possible, but at least some species of *Bufo* (those of the *debilis* group) hatch somewhat later. Similarly, in other genera the stage at hatching varies interspecifically, as Moore (1940) observed for *Rana* of the northeastern United States. In addition, there is variation among clutches of the same species, and even within one clutch all embryos may not attain the same level of development before hatching. Moore (1940) attributed adaptive significance with respect to water temperature and availability of dissolved oxygen to the stage at which hatching occurs, and Grainger (1959) observed that in *Rana temporaria* the stage at hatching varies with the temperature at which the eggs are raised.

Moore (1940, pp. 91–92) based his observations on the relationship between stage at hatching and temperature on species of *Rana;* some of these deposit their eggs in very cool water, whereas others breed in warmer water. He noted that the need of the embryo for oxygen increases greatly as development proceeds, and the late embryos of a species breeding in warmer water would be in a better position to obtain sufficient oxygen if hatched and free of the egg membranes, particularly if the eggs are laid in a globular mass. Moore found that *Rana sylvatica* (upper limiting temperature, 24° C.) hatches late in stage 20 or early in stage 21, *R. pipiens* (28° C.) in late stage 17 to 18, and *R. palustris* (30° C.) in stage 17. These three deposit globular egg masses. Two species that deposit the eggs in a surface film, *R. catesbeiana* and *R. clamitans*, hatch in stage 17 or early stage 18 and have an upper limiting temperature of 32° C.

The stage at hatching of the various anurans in the Chiricahua region is discussed in the accounts of development rates, so the details are not repeated. The order of species with respect to earliness of hatching is (usual stage given in parentheses): *Bufo cognatus* (16); *B. punctatus* (17); *Scaphiopus couchii* (18); *B. debilis* (19); *S. bombifrons*, *S. hammondii* and *Rana pipiens* (19L–20); *Hyla arenicolor* (20L). A correlation is indicated here between stage at hatching and adaptation to temperature, as measured by

the upper limiting temperature. *Hyla arenicolor*, *Rana pipiens*, *Scaphiopus hammondii*, and *S. bombifrons* have the lowest upper limiting temperatures among the species studied and are those that hatch latest. *Scaphiopus couchii* has the highest upper limiting temperature of any of the species studied and hatches at a relatively early stage.

The only two species conspicuously out of place in the foregoing sequence are *Bufo punctatus* and *Bufo cognatus*. Members of the genus *Bufo* apparently are exceptions to the rule that species adapted to higher temperatures hatch at earlier stages than those adapted to lower temperatures. The difference is seen most clearly in a comparison of the tropical species *Bufo valliceps* (upper limiting temperature 35° C.) with *Bufo americanus* of northeastern North America (upper limiting temperature 31° C.); both hatch at a typically early stage (Limbaugh and Volpe, 1957, p. 6).

Grainger's (1959) observation that embryos of *Rana temporaria* raised at lower temperatures tend to hatch at a later stage than those raised at higher temperatures is in line with Moore's reasoning as to the significance of variation in the stage at which hatching occurs. It is perhaps worth reiterating that instances of variation in stage of hatching that I recorded in *Bufo cognatus* were not obviously correlated with temperature (see foregoing account of development rate of *B. cognatus*).

BREEDING BEHAVIOR

Some species are rather restricted in breeding sites and in time of breeding by factors that may have little or nothing to do with temperature. The adults may be confined to a particular habitat, or they may be capable of breeding only during a restricted period. On the other hand, the capability of members of a population to breed over several months of the year and their not being narrowly restricted in habitat selection, both clearly the case in *Rana pipiens*, can compensate for apparent deficiencies in embryonic adaptation to temperature. Populations with narrow spans of tolerance (18–19° C.), low upper limiting temperatures (29–31.5° C.), and slow developmental rates (hence slow expansion of the span of tolerance) live in such different locales as the Chiricahua Mountains and in the desert 125 feet below sea level in southern California (Ruibal, 1959, 1962). *Rana pipiens* adapts to the desert climate by breeding in winter; in the more equable climate of the Chiricahua Mountains, the breeding season extends throughout much of the year. A species without such behavioral adaptability could not show such a wide ecological distribution unless the embryos were more broadly adaptable to different temperatures.

TEMPERATURE TOLERANCE IN RELATION TO GEOGRAPHIC DISTRIBUTION

THE DISTRIBUTION, both in the broad geographic sense and in the narrower ecological sense, of a species results from the interaction of a multitude of factors intrinsic and extrinsic to the individual organisms. Nevertheless, sometimes one or a very few limiting factors may dominate. It is useful, therefore, to examine the temperature tolerances and developmental rates as possible limiting factors.

While acknowledging that "eliminating all other factors because they are either unknown or improbable [was] a most dubious procedure," Moore (1949b, p. 325) considered "temperature as the principal known factor responsible for the northern and southern limits of distribution of the wide-ranging eastern American" species of *Rana*. He reasoned that, because the embryonic stages show the least tolerance for temperature extremes, temperature would most likely act as a limiting factor in this part of the life cycle. His investigations did reveal clear correlations between temperature adaptations on the one hand and geographic distribution on the other. The influence of habitat selection and breeding season (e.g., delay of breeding into summer by "southern" species living in the north) was not sufficient to obscure the correlations.

In the broad picture of their geographic distribution, the frogs of the Chiricahua region do not show the clear correlations between limiting temperatures and distribution seen in *Rana* of the eastern United States. The western frogs not only inhabit a region of greater environmental complexity but are themselves quite a diverse group of species, in contrast to the congeneric assemblage studied by Moore, but correlations are not lacking.

The spadefoot toads *Scaphiopus couchii* and *S. hammondii* are broadly sympatric throughout much of their ranges, but their range limits differ conspicuously on the west and somewhat less so at the north. *Scaphiopus hammondii* evidently is absent from the low deserts of western Arizona and southern California but reappears west of the deserts of California. *Scaphiopus couchii* occurs in western Arizona and arid southeastern California (Mayhew, 1965), and also in the hot and somewhat moister lowlands of Sonora, Sinaloa, and Nayarit. The northern range limits of *hammondii* lie to the north of those of *couchii*.

The ability of *S. hammondii* to invade coastal California and the failure of *couchii* to exist there may readily be explained in terms of the tolerance of embryos for low temperature. In the Mediterranean climate of the California lowlands, rainfall is almost totally restricted to cool periods in winter and early spring; there are no summer rains. Rarely, if ever, is there rain of such intensity and warmth that it would promote breeding by *couchii*, but *hammondii* is less inhibited by low temperatures. It appears that *hammondii* of southern California may have gone even further then *hammondii* of Arizona in adapting to low temperatures, for Brown (1967, p. 366) reported that embryos of *hammondii* of Arizona exhibit higher temperature-tolerance limits than those of *hammondii* from southern California. The penetration of *hammondii* northward into parts of Colorado and Oklahoma not reached by *couchii* may also be related to temperature tolerance, but there the relationship between rainy season and temperature is not present.

The presence of *S. couchii* in hot lowland areas where *hammondii* is absent may be related to the higher upper limiting temperature of *couchii*. An additional possible factor, at least as far as the area of western Mexico is concerned, is the presence there of *Pternohyla fodiens*. This burrowing hylid frog has habits much like those of a spadefoot toad and may occupy an ecological niche closely resembling that of *hammondii*.

No correlations such as those found in the distributions of *Scaphiopus couchii* and *S. hammondii* are evident with regard to *S. bombifrons*. The last species is primarily a species of the Great Plains, and its distribution may reflect its adaptation to the grassland habitat more than limitations imposed by temperature.

I can detect no correlations between range limits and temperature tolerance in the three species of *Bufo* studied. *Bufo debilis* might be expected to range southward into Sonora and Sinaloa, but it is replaced in western Mexico by two members of the same species group, *B. kelloggi* and *B. retiformis*. *Bufo punctatus* may be limited in range by its predilection for breeding (though not invariably) in canyon streams and potholes. The coolness of these habitats may limit the northward extent of its range; recall that, in a desert canyon in southern California, *punctatus* evidently breeds at temperatures low in the range of tolerance (Tevis, 1966; see the foregoing discussion under Temperature Tolerances in Relation to Environmental Temperatures). *Bufo cognatus* shows a wide distribution, possibly in part a reflection of its adaptability with regard to breeding season.

Embryos of *Hyla arenicolor* are perhaps the least tolerant of high temperatures among the several species studied, yet the southward range of the species is matched only by *Scaphiopus hammondii* (both reach the southern end of the Mexican Plateau). The relative coolness of canyon streams, especially at high elevations, may permit *arenicolor* to range far to the south and may also (as in *Bufo punctatus*) inhibit northward expansion.

Because of the variation *Rana pipiens* as a whole exhibits in temperature tolerance, and because of the confusion as to whether one or more species are involved in the complex, there is little point in prolonging this discussion of the Chiricahua population. It should be emphasized, however, that, although this population of *pipiens* exhibits a rather narrow range of embryonic temperature tolerance, the apparent disadvantages are offset by adaptability with respect to breeding season. At least some members of the population are capable of breeding throughout most of the year; thus the population can take advantage of seasonal changes in water temperature much more readily than could one with less flexibility.

Gastrophryne olivacea, a species that does not occur in the Chiricahua region, is worthy of brief discussion. It is present in south-central Arizona and Sonora but has not been found north of the border in eastern Arizona. Data presented by Ballinger and McKinney (1966) indicate that the lower limiting temperature for early embryos of this species is about 19° to 20° C. *Gastrophryne* typically breeds following heavy rains, which in my study area usually fall at temperatures around 20° C. It is probably true that the lowest temperature at which frogs will initiate oviposition is slightly above the lower limiting temperature for early embryonic development. *Scaphiopus couchii*, for example has a lower limiting temperature of 15.5° C. and evidently does not breed unless the temperature is about 18° C. or higher. Thus, conditions may be too cool when it rains in the Chiricahua region to stimulate breeding by individuals of *Gastrophyne* and to permit the species to extend its range northward, and maintain populations there.

SUMMARY

NINE SPECIES OF ANURAN AMPHIBIANS inhabit desert-grassland and semiarid uplands of southeastern Arizona and adjacent New Mexico in the vicinity of the Chiricahua Mountains. Embryos of eight of these were raised under constant temperature conditions in order to determine rates of development and upper and lower temperatures limiting for normal development.

The species most tolerant of high temperatures is *Scaphiopus couchii*, early embryos of which can develop normally at a constant temperature of 34° C. *Rana pipiens*, with an upper limiting temperature of 31.5° C., may be least tolerant of high temperatures, although the limit for *Hyla arenicolor* may be even lower. Greater differences exist with respect to low temperatures. *Bufo debilis* may be least able to withstand low temperatures, but its lower limit was not established with precision. *Bufo cognatus* is unable to develop normally below 16° C., and *B. punctatus* probably has approximately the same limit. In contrast, *Rana pipiens* probably can develop at a temperature as low as 12° C., and the limit for *Scaphiopus hammondii* may be even lower.

The temperatures limiting embryonic development correlate with conditions in the breeding sites. *Scaphiopus couchii*, with the highest upper limiting temperature, breeds in temporary desert rain pools that are the warmest aquatic habitats. *Rana pipiens* and *Hyla arenicolor*, both tolerant of cool waters, inhabit mountain streams. The situation is by no means clear-cut, however, and other factors in addition to temperature tolerance figure importantly in the selection of breeding sites.

Rates of embryonic development differ greatly among the eight species. The three species of *Scaphiopus* exhibit rapid rates at all temperatures, but *S. couchii* develops more rapidly at high temperatures, and *S. hammondii* and *S. bombifrons* develop faster at low temperatures. The three species of *Bufo* and *Hyla arenicolor* all show similar rates, markedly slower than those of *Scaphiopus*. *Rana pipiens* is by far the slowest of all except at temperatures approaching the minimum tolerated by *Bufo*, at which *pipiens* develops at a similar or slightly more rapid rate.

The effect of change in temperature on developmental rate (the slope of the rate curve) differs in the same fashion. A given change in temperature has roughly the same effect on the three species of *Scaphiopus*, and on *Bufo* and *Hyla* (most on *S. couchii*, least on *H. arenicolor*), and the least effect on *Rana pipiens*. An increase of 10° in temperature, from 19° to 29° C., increases the rate of development of most of the species about three to four times, but only doubles that of *pipiens*.

The most rapid embryonic development is found in the three species of *Scaphiopus* that breed in temporary desert rain pools. The species of *Bufo* and those of *Hyla* have less rapid rates of development and normally utilize breeding sites where the water supply is more lasting but not necessarily permanent. The slowest developer, *Rana pipiens*, apparently breeds only in permanent waters. Thus there is a correlation between rate of development and water supply.

Rapid development may be particularly advantageous to frogs breeding in warmer habitats because of the increase in the range of temperature tolerance of the embryos that accompanies development. The upper limiting temperatures of embryos of *Scaphiopus bombifrons* and *S. hammondii* are rather low early in development, but development is so rapid that the most heat-sensitive stage is likely to be passed before the morning sun warms the ponds to dangerously high levels. *Scaphiopus couchii*, with an initially high level of temperature tolerance and even more rapid development, is perhaps the best adapted to high temperature among all species studied.

Other features of the embryonic stages that may reflect adaptation to temperature include the color and size of the ovum, the form of the egg mass, and the stage of development of the embryo when it hatches. To a considerable extent these features are interrelated. A compact mass of dark eggs exposed to sunlight becomes warmer than surrounding still water, an advantage under cool conditions but disadvantageous in warm water. Embryos in cool water, even in a compact mass, are likely to be exposed to a higher oxygen tension than those in warm water and can develop to a later stage, at which the demand for oxygen increases greatly, before hatching. Among the species studied, only *Rana pipiens* lays dark eggs in a

compact mass from which the embryos hatch at a late stage of development.

The remaining species vary greatly in the manner in which the eggs are deposited, but, whether they are arranged singly, in long strings, or in small masses, the eggs are well exposed to the water. Presumably this reduction or lack of clumping is necessary in the low oxygen tension of warm water, and also avoids the concentration of dark pigment that presumably is partly responsible for the differential heating of a compact mass. Other factors may be of greater importance than adaptation to temperature in influencing the form of the egg mass. Eggs deposited singly may have greater survival value in a running-water habitat (less likely to be dislodged, or less conspicuous to predators). Both *Bufo punctatus* and *Hyla arenicolor* breed in running water, or where pools may suddenly be scoured by floods, and both produce eggs singly, at least part of the time. The long strings of eggs produced by most species of *Bufo*, but only by *B. cognatus* in the study area, evidently are well adapted both to cool and warm still-water habitats.

If dark coloration of an ovum promotes absorption of heat, lighter eggs might be anticipated in species adapted to warm-water conditions. Dark pigment, however, may also be of importance in protecting the embryo from injury by ultraviolet radiation, so the relationship is not simple. Related species breeding in the same pond at the same time (*Scaphiopus couchii* and *S. hammondii*) have eggs that differ noticeably in the amount of dark pigment.

Rana pipiens and *Hyla arenicolor* hatch relatively late in development (stage 20), usually after the gills have started to function. Such late hatching in species adapted to relatively cool conditions agrees with the presumption that the greater oxygen supply in cool water permits a longer period of development within the egg membranes. Similarly, *Scaphiopus couchii* hatches earlier (stage 18) than *S. bombifrons* or *S. hammondii* (late stage 19 or stage 20) and is adapted to warmer temperature than the last two. *Bufo cognatus* and *B. punctatus* conform to the usual *Bufo* mode of hatching at a very early stage (stage 16 or stage 17), regardless of other indications of adaptation to temperature, whereas *Bufo debilis* hatches rather late (stage 19). Therefore, although there are indications of correlation between stage at hatching and adaptation to temperature, the relationship is not a critical one.

For the most part, there are no strong correlations between the geographic distribution of the species studied and the adaptations of their embryos to temperature. A possible relationship of temperature tolerance to distribution is seen in the species *Scaphiopus couchii* and *S. hammondii*. The former ranges eastward from Arizona into the hot desert of southeastern California but does not occur on the Pacific coast. *Scaphiopus hammondii* does not occur in the California deserts but is present in coastal California. Rainfall in the coastal region is almost totally limited to winter and early spring, when temperatures are suitable for the relatively cold-tolerant *hammondii* but too low for the early embryos of *couchii*. These spadefoot toads are not restricted to breeding at any one time of the year, but are limited to breeding in temporary rain pools. This inflexibility of their breeding habits may give temperature a more important role in determining the distribution of *Scaphiopus* than is the case in other species that can vary both breeding season and habitat to some extent.

To summarize, the adaptation of frog embryos to temperature takes a variety of courses. Basically, each embryo in its earliest stages of development is capable of tolerating temperatures within a range characteristic of the species or at least of the population. Typically, this range differs from species to species. The range of tolerance expands as the embryo develops, and a rapid rate of embryonic development may be regarded in part as an adaptation to temperature. Features adaptive to high temperature include laying eggs in small masses and hatching at a relatively early stage of development; conversely, very dark eggs in large masses absorb heat more readily and are thus adapted to cooler waters. Superimposed upon the adaptations of the embryos are adaptive aspects of the breeding habits of the frogs, notably habitat selection and variation in time of breeding. The interactions of these various features permit the coexistence of species that differ from one another, in some cases markedly, in one or more aspects of adaptation to temperature.

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