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TEMPERATURE TOLERANCE AND RATES OF DEVELOPMENT IN THE EGGS OF AMPHIBIA ¹

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Although the effect of temperature on the rate of embryonic growth in Amphibia was known before the turn of the last century, it remained for Lillie and Knowlton ('97) to supply the first quantitative data on the subject. They found that as the temperature was increased above the lowest at which eggs of *Ambystoma tigrinum* or *Rana pipiens* can develop, the time necessary to reach a given morphological stage becomes progressively less, until a temperature is reached above which normal development does not occur. It was also recognized that the time-temperature curve resembles those "measuring the rapidity of known chemical processes at different temperatures." In view of the work to be presented, it is of interest that they found the temperature tolerance of eggs to be correlated with environmental conditions. The eggs of species breeding early in the year when the water was cold, could tolerate a lower temperature than could those of forms breeding later in the spring. This paper of Lillie and Knowlton was followed by the masterly investigations of Hertwig in 1898, and others such as those of Galloway ('00), King ('03), Chambers ('08), Krogh ('14), Dempster ('33), Atlas ('35), and Knight ('38).

The present investigations are concerned with a comparison of the effects of temperature on the embryos of different amphibians. The temperature tolerance and rates of development, over a wide range of temperatures have been determined in four species of frogs. A comparison of developmental rate among four species of salamanders and between two toads has been made. Correlations between temperature tolerance and rates of development on one hand, and breeding habits and geographic distribution on the other, have led to certain conclusions regarding adaptation to environmental temperature

¹ It is a pleasure to acknowledge my indebtedness to Professor Barth who has directed these investigations, and to Professors Pollister and Barth for allowing me to use their data on rate of development in *Rana catesbeiana*. These investigations were aided in part by a grant in Chemical Embryology from the Rockefeller Foundation administered by Professor Barth.

conditions in these forms. A preliminary notice of these experiments has appeared (Moore, '38).

The Anuran genus *Rana* is represented by six species in New England and eastern Canada, namely: *R. sylvatica* Le Conte (in eastern Canada replaced by *R. s. cantabrigensis* Baird, according to Trapido and Clausen, '38), *R. pipiens* Schreber, *R. palustris* Le Conte, *R. clamitans* Latreille, *R. catesbeiana* Shaw, and *R. septentrionalis* Baird. The genus *Bufo* is represented by *B. americanus* Holbrook, and *B. fowleri* Hinckley, and the urodele genus *Ambystoma* by *A. tigrinum* (Green), *A. opacum* (Gravenhorst), *A. jeffersonianum* (Green), and *A. maculatum* (Shaw). These species differ in their geographic distribution and time of laying, with correlated differences in environmental temperatures to which the eggs are subjected. They form excellent material for studying adaptation to temperature differences. Data have been collected on all the above mentioned species with the exception of *R. septentrionalis*.

METHODS

The discovery that the ovaries of mature frogs can be stimulated to release their eggs by injecting the anterior lobe of the pituitary gland (Wolf, '29; Rugh, '34), has simplified the securing of eggs in early stages for temperature analysis. There is little difficulty in collecting eggs of *R. sylvatica* before first cleavage, as they are deposited when the water is cold and development slow, but this becomes increasingly difficult in the cases of species breeding later. It is with the latter that the pituitary technique has been most helpful. No differences have been detected between freshly collected eggs and those secured by injections. *R. sylvatica*, *R. pipiens*, and *R. palustris* collected in the autumn, produce eggs that give over 90 per cent fertilization and normal development when injected with *R. pipiens* pituitary glands. *R. clamitans* has not been injected in the autumn, but such treatment is successful in securing eggs in the spring before the normal breeding season.

The general procedure for frogs (*Ambystoma* and *Bufo* eggs were collected under natural conditions) was to fertilize the eggs artificially, and allow the jelly to swell. The egg mass was then cut into groups containing from 1 to 10 eggs, and several of these groups placed in finger bowls with 0.1 Amphibian Ringer's solution. Shortly before the onset of first cleavage, these bowls were placed at the desired temperatures. The eggs were examined at subsequent times, and the temperature of the 0.1 Ringer's and the stage of development recorded. Temperature control was by means of water-baths with mercury thermo-regulators, incubators, cold rooms, and refrigerators. Temperature measurements are in degrees centigrade.

The stages of development defined by Pollister for *R. sylvatica* (Pollister and Moore, '37) have been employed for all frogs (*Rana*) and toads (*Bufo*). Harrison's unpublished stages for *Ambystoma punctatum* have been used for all members of this genus.

The observations on rate of development are arranged in tables and plotted graphically. Thus Table I gives the time at which eggs of *Rana sylvatica* were observed in different stages at a variety of temperatures. When two figures are given they represent the earliest and latest hours at which eggs were observed in a particular stage. Thus at $15.3 \pm 0.3^\circ$ they are in stage 14 by 54 hours after first cleavage (stage 3). At 60 hours they are still in stage 14. Sometime between 60 and 63 hours they enter stage 15 but no observations have been made during that interval. The data in the $15.3 \pm 0.3^\circ$ column are composed of observations on eggs from two different females. One experiment was begun March 21, 1936, and the temperature was $15.4 \pm 0.2^\circ$. The second experiment was begun March 19, 1938, and the temperature was $15.1 \pm 0.1^\circ$. The number of females used in compiling the data in each column is indicated under "experiments" in the tables.

The graphs (Figs. 1-4) are constructed to simplify comparisons of the effect of temperature on rate of development. The data from one temperature are plotted as a straight line by varying the intervals between stages on the abscissa. Using this straight line as a base the data from other temperatures are then plotted. It is easier to compare the results at different temperatures when the base line is straight and the intervals on the abscissa varied, than when the latter are made uniform with a resulting irregular curve.

EXPERIMENTS

Rana sylvatica. The wood frog is the first member of its genus to breed. In spring shortly after the melting of the ice (usually the middle of March in the vicinity of New York City) the adults collect in woodland pools where their eggs are deposited in a firm oval jelly mass, in water that averages 10° . The wood frog is the most northerly distributed of our eastern frogs. In Canada it is represented by the closely related variety, *R. sylvatica cantabrigensis* (see Trapido and Clausen, '38). This, or a similar form, has been taken as far north as $67^\circ 30'$ on the Mackenzie River by Preble ('08).

In view of the early breeding habits and northern distribution one might expect the eggs of this form to tolerate extreme cold, and indeed this is so. It has been customary to keep the eggs at $2.5 \pm 1.0^\circ$ and subsequently use the embryos in transplantation experiments. Eggs placed in the cooling unit of the refrigerator for 12 hours developed normally after being returned to 20° even though a layer of ice had formed over the water. Although the eggs of this species can tolerate a lower temperature than those of any other frog investigated, they are the least resistant at higher temperatures. Eggs from four different females developed normally at $23.7 \pm 0.2^\circ$. Eggs from another gastrulated abnormally at $23.6 \pm 0.4^\circ$. Still another group died in stage 16 when kept at $24.5 \pm 0.2^\circ$. Finally at $27.2 \pm 0.2^\circ$ eggs from four different females died as blastulae.

TABLE I. Time in hours after first cleavage that eggs of *Rana sylvatica* were in various stages at different temperatures

Experiments	2	2	1	3	1
Temperature	10.0±0.6°	15.3±0.3°	18.5±0.2°	19.9±0.1°	23.7±0.2°
Stage 3	0	0	0	0	0
8	24	14-18.5		14.5	9
9	36	21-23	14-16		
10	48-52	24-29	17-18	16-20	
11	60-66	35	22-24	20-23	
12	72-84	37-48	26-30	23-26	17.5-21
13	96-114	47-50.5	34	37.7	
14	120-132	54-60	38	37.7	29
15	137	63-66		40.5	
16	144-162	67.5-73	49.5	40-45	
17	168-171	77-90.5	52.5-54		35
18		84-101	57-70	50	41-43
19	228-304	108-113	73	66	47-50
20	282-327	123-132	87	72-87	52

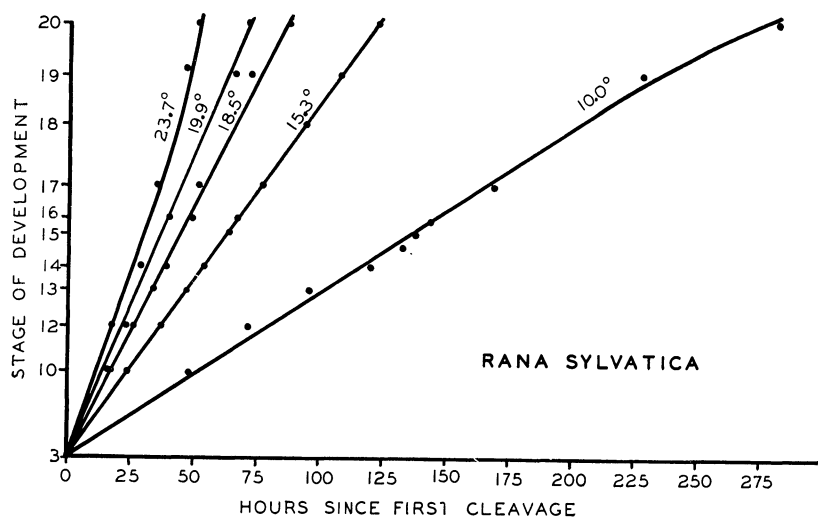


FIG. 1. The relation between temperature and rate of development in *Rana sylvatica*.

The eggs of this species develop (Fig. 1, Table I) more rapidly than do those of any other form studied. This rapid development is continued, and in from 50 to 60 days (maximally fed with egg yolk and spinach and kept at 20°) after first cleavage the front legs of the metamorphosing tadpoles appear. Adolph ('31) finds that at 19° the forelegs are acquired in 54 days. Under natural conditions from 61 to 115 days are required (Wright, '14).

Rana pipiens. This species generally breeds early in April near New York City (the best description of the breeding habits of the frogs used in

these experiments is to be found in Wright, '14). The temperature of water where the eggs are shed, averages 12° (Wright, '14). Preble ('08) found *R. pipiens* as far north as the sixtieth parallel in the region near Fort Smith. While existing records leave much to be desired, it appears that *R. pipiens* is found farther north than *R. palustris*, *R. clamitans*, or *R. catesbeiana*. It is not distributed as far north as *R. sylvatica*.

The eggs of *R. pipiens* used in these experiments averaged 1.7 mm. in diameter. They are somewhat smaller than those of either *R. sylvatica* or *R. palustris*. The oval jelly mass in which they are deposited lacks the firmness of that in the two latter species.

TABLE II. Time in hours after first cleavage that eggs of *Rana pipiens* were in various stages at different temperatures

Experiments	5	2	12	4
Temperature	15.3±0.1°	18.6±0.1°	19.8±0.2°	26.0±0.4°
Stage 3	0	0	0	0
4		1		
5		2		
8	13.5-21.7			7
9	24-29	19-20.5		
10	36-40	22-24	20-24	13.5
11	43.5	28-30	25	
12	48-59.5	32-41.5	29-37.5	16.5-19
13	67-68.2	45.7	38-44	21.5-24
14	73-84	48-54	43.5-48	27.5
15			50-54.5	
16	88-96.5	60-65	51-58	29
17	117-123.5	68-73	60-69	36-41
18	142	77-102	70-84	43-48
19	149-163	103-114	85-96	50-51
20	168	116	95-103	54-59

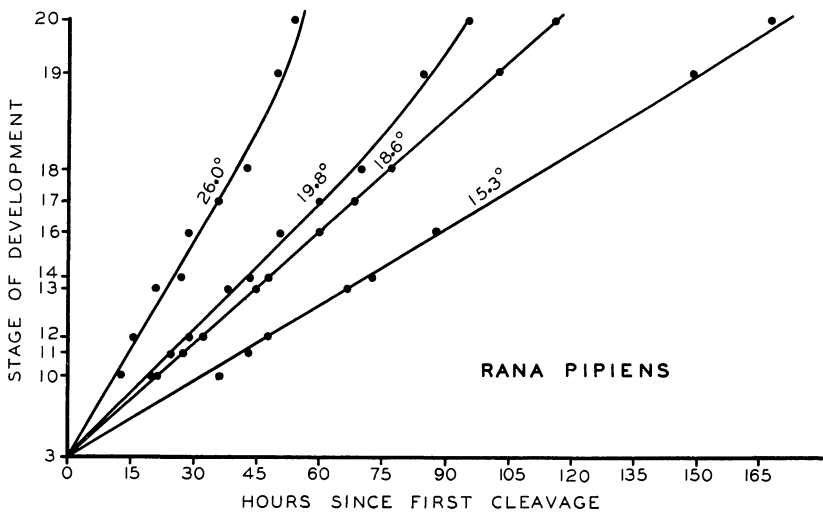


FIG. 2. The relation between temperature and rate of development in *Rana pipiens*.

The eggs are killed by temperatures as low as $2.5 \pm 1.0^\circ$. They cannot, therefore, tolerate as low a temperature as *R. sylvatica*. When kept at $5.0 \pm 1.9^\circ$ from stage 3 to 10, cleavage in the vegetal hemisphere was irregular, but in spite of this they developed into normal tadpoles when removed to 20° for the remaining time. Normal development occurred when the eggs were kept at $8.4 \pm 0.5^\circ$. Atlas ('35) found 5° to be subminimal.

The upper limiting temperature is approximately 28° . At $29.1 \pm 0.4^\circ$ over half of the eggs from one female developed abnormally. All those from another female formed abnormal tadpoles at $29.6 \pm 0.9^\circ$. In yet another case, however, perfectly normal tadpoles were produced at $30.0 \pm 0.3^\circ$. This latter case was probably exceptional as both Atlas ('35) and Hoadley and Brill ('37) find 29° supramaximal for development.

The effect of temperature on developmental rate in *R. pipiens* has been admirably treated by Atlas ('35). Since he did not use the same stages that are employed here, this form has been reexamined (Table II and Fig. 2). Development to stage 20 is 32 per cent slower than in *R. sylvatica* at 19.9° . The time required from egg to transformation at 19° is 117 days (Adolph, '31). Under natural conditions this occurs in from 71 to 111 days (Wright, '14).

Rana palustris. The next in order of breeding is the pickerel frog. Near New York City breeding occurs about the middle of April. Pond water at this time averages 14 to 16° (Wright, '14). This species has been taken at James Bay ($51\text{--}54^\circ$ N., Cope, '89). It was not recorded by Preble from west of Hudson Bay ('02), or in the Athabaska-Mackenzie region ('08), so evidently it does not range as far north as *R. pipiens* or *R. sylvatica*. The eggs average 1.85 mm. in diameter and are deposited in a firm oval jelly mass.

TABLE III. Time in hours after first cleavage that eggs of *Rana palustris* were in various stages at different temperatures

Experiments	2	2	2	2	1
Temperature	$15.5 \pm 0.2^\circ$	$18.6 \pm 0.1^\circ$	$19.9 \pm 0.2^\circ$	$25.7 \pm 0.5^\circ$	$30.4 \pm 0.4^\circ$
Stage 3	0	0	0-1	0	0
9	29	19-22		11.5	
10	35-43	24-30	20-24	13	11
11	51	32	29.5		
12	49-69	36-45	35	20-21	21
13	66-74	45-50	38-43	24	
14	80-92	52-60	46-55	30	24
15	96-98		55		
16	107	65-68	61-64	34.5-37.5	
17	103-138	73-78	66-74	42-43	34-42
18	138-170	89-114	80-83	49-52	48
19	167-180	119-124	95-98	56	
20	187-192	126	105-106	60-66	
21					60

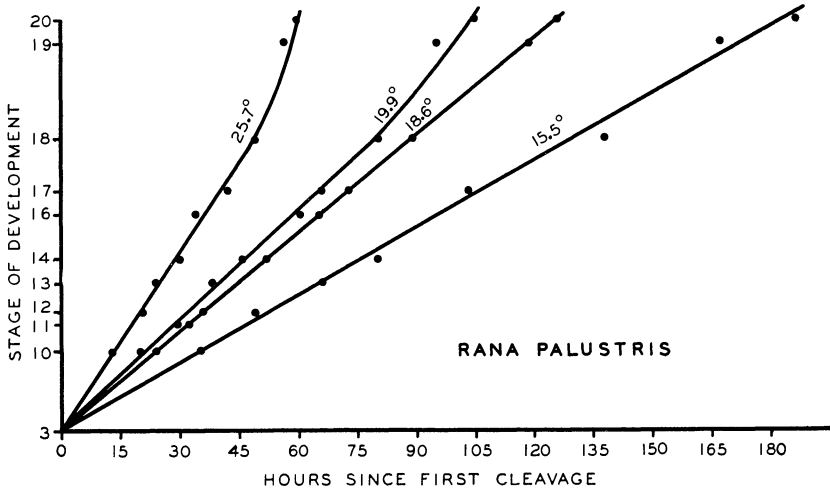


FIG. 3. The relation between temperature and rate of development in *Rana palustris*.

As in the case of *R. pipiens* eggs kept at $2.5 \pm 1.0^\circ$ are killed. Those in one experiment were killed at $5.8 \pm 0.7^\circ$. Normal development occurs at $8.6 \pm 0.5^\circ$. The maximum is near 30° . At $30.4 \pm 0.4^\circ$ most of the eggs from one female developed normally. At $31.9 \pm 0.1^\circ$ all died before gastrulation. The limits of temperature tolerance are similar to those of *R. pipiens*, but appear to be a degree or so higher. Neither *R. palustris* nor *R. pipiens* can withstand temperatures as low as *R. sylvatica*. The maximum of *R. palustris* is near 30° , compared with 28° for *R. pipiens*. However there is some overlapping as in one case the latter developed normally at $30.0 \pm 0.3^\circ$.

Development to stage 20 is 10 per cent slower than in *R. pipiens*, and 46 per cent slower than in *R. sylvatica*. The data for development are in Table III and figure 3. Under natural conditions the time from egg to metamorphosis is 90 to 100 days (Wright, '14).

Rana clamitans. The green frog, *R. clamitans*, breeds in late spring and early summer when the temperature of pond water averages 25° (Wright, '14). It does not extend as far into Canada as the earlier breeding species, but it has been taken as far north as Lake Nipigon, Ontario (50° N.) by Logier ('28), and in the Lake Abitibi region (49° N.) by Dymond ('28). The eggs used in these experiments averaged 1.4 mm. in diameter. They are normally deposited in a surface film, rather than in the partially or completely submerged compact mass characteristic of the early spring breeders.

Eggs kept at $4.7 \pm 0.3^\circ$, and at $10.0 \pm 0.3^\circ$ were killed before gastrulation. At $12.2 \pm 0.5^\circ$, and temperatures up to and including $33.4 \pm 0.2^\circ$, normal development takes place. At $36.1 \pm 0.3^\circ$ the eggs die as blastulae. The upper limiting temperature therefore is near 35° .

The rate of development (Table IV; Fig. 4) is slower than in any

TABLE IV. Time in hours after first cleavage that eggs of *Rana clamitans* were in various stages at different temperatures

Experiments		2	1	2	1
Temperature		15.0±0.3°	19.8±0.2°	25.3±1.0°	33.4±0.3°
Stage	3	0	0	0	0
	7	6			
	8	18-32			
	9			6	
	10	44-50	20-22		
	11	50-55	25		
	12	56-80	33	18-22	
	13	93		23.5	
	14	97	43-50	26.2	
	15	109		28	
	16	111-125	53-56	32	22
	17	141-164	67-72		
	18	164-200	73-93	42-45	31
	19	215-222	97-111	50-56	
	20	238-287	112	58-65	45

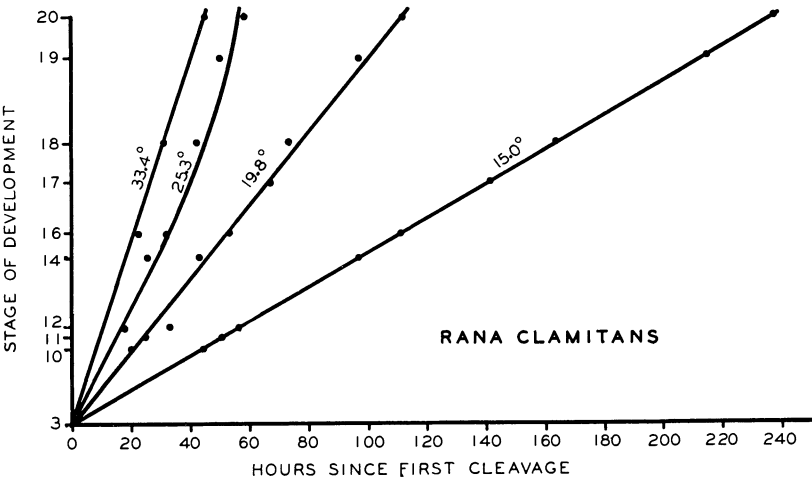


FIG. 4. The relation between temperature and rate of development in *Rana clamitans*.

species treated previously. At 19.9° development to stage 20 is 57 per cent slower than in *R. sylvatica*, 19 per cent slower than *R. pipiens*, and 7 per cent slower than *R. palustris*. Under natural conditions *R. clamitans* does not transform until one year after the beginning of development. This is in marked contrast to *R. sylvatica*, *R. pipiens*, and *R. palustris* all of which transform in several months.

Rana catesbeiana. The bullfrog, *R. catesbeiana*, is the last member of its genus to breed in this region. Correlated with this, its northern distribution is less extensive than is that of any of the forms previously described. It is absent from the Gaspé Peninsula (48° N.), Lake Nipigon (50° N.,

Logier, '28), and Lake Abitibi (49° N., Dymond, '28). Toner and Edwards ('38) found it common at Grippen Lake, Leeds County, Ontario (44° 30' N.). Cox ('98) found it in New Brunswick, and Coventry ('31) reported it from the Temgami District (47° N.).

The eggs are similar to those of *R. clamitans* in size and are likewise deposited in a surface film.

TABLE V. *Hours of development of the eggs of Rana catesbeiana, Bufo americanus, and Bufo fowleri in the various stages*

	<i>Rana catesbeiana</i>	<i>Bufo fowleri</i>	<i>Bufo americanus</i>
Experiments	1	1	1
Temperature	18.5°	19.9±0.2°	19.9±0.1°
Stage 3		0	
7			0
8	0-4	10.5-12.5	
10	15-16.5	17	13
11	16.5-21		
12	24	22	16.5-21
13	34.5		27
14	50		39
16	63.5		45
17	73	58	61
18	88-113	70	
19	118-149	94	74
20	159	107	85

No observations have been made on the temperature tolerance of the eggs of this form. The data on development (Table V) were gathered by Dr. Pollister and Dr. Barth. They have very kindly allowed me to use their observations to supplement the material already presented. They found the time from stage 8 to 20 to be 159 hours at 18.5°. At this temperature 170 hours would be a fair estimate of the time from stage 3 to 20. With this value we can compare the rate of development with that of the other forms (as read from the curve in figure 6). *R. catesbeiana* is then 95 per cent slower than *R. sylvatica*, 46 per cent slower than *R. pipiens*, 35 per cent slower than *R. palustris*, and 27 per cent slower than *R. clamitans*. *R. catesbeiana* has the longest larval period of any species in northeastern North America. The tadpole does not transform until the second summer after the beginning of development (Wright, '14).

COMPARISONS

In the previous section it was shown that those frogs characteristic of northern regions, and which breed early in the year when environmental temperatures are low, differ from species characteristic of more temperate conditions as follows:

1. In being able to tolerate lower temperatures in the egg and larval stage, and being more susceptible to higher temperatures.
2. In compensating for the retarding effect of the low temperature of their environment by a rapid rate of development.

The limits of temperature tolerance are shown in figure 5. It will be seen that the eggs of all four species are not able to develop over the same wide range of temperatures, but each species is adapted to a narrower range which covers the conditions existing in its particular environment at the time of breeding. It is also of interest that the number of degrees over which normal development occurs (difference between the upper and lower limiting temperatures) is practically identical, being 22–24°.

A comparison of the rates of development of these same four species is made in figure 6. *R. sylvatica* which is best adapted to cold with respect to temperature tolerance in early stages, has the most rapid rate of development. Species breeding later when the surrounding pond water is warmer develop progressively slower. It will be seen from the curves that the difference in rate is most apparent at low temperatures. This will be discussed later.

A summary of the various correlations so far presented is given in Table VI. The values for the upper and lower limiting temperatures are somewhat approximate. Thus in the case of *R. clamitans*, the lower limit for development is placed at 11°, as 10.0° is too low for normal development, but 12.2° is quite suitable. Likewise these same eggs develop at 33.4° but are killed at 36.1°. The upper limiting temperature is then placed at 35°.

RESULTS ON OTHER GENERA

The fact that the range of temperature tolerance of frog eggs is similar to the environmental temperatures to which these eggs are subjected in nature

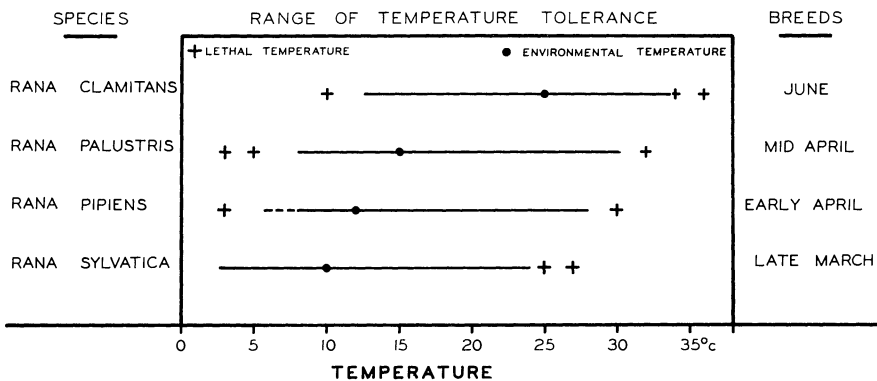


FIG. 5. The relation between temperature tolerance and breeding habits in frogs. The heavy black lines indicate the temperatures at which normal development takes place. Lethal temperatures are indicated by crosses. The average water temperature at the time of egg-laying is indicated by the dot on the temperature tolerance line.

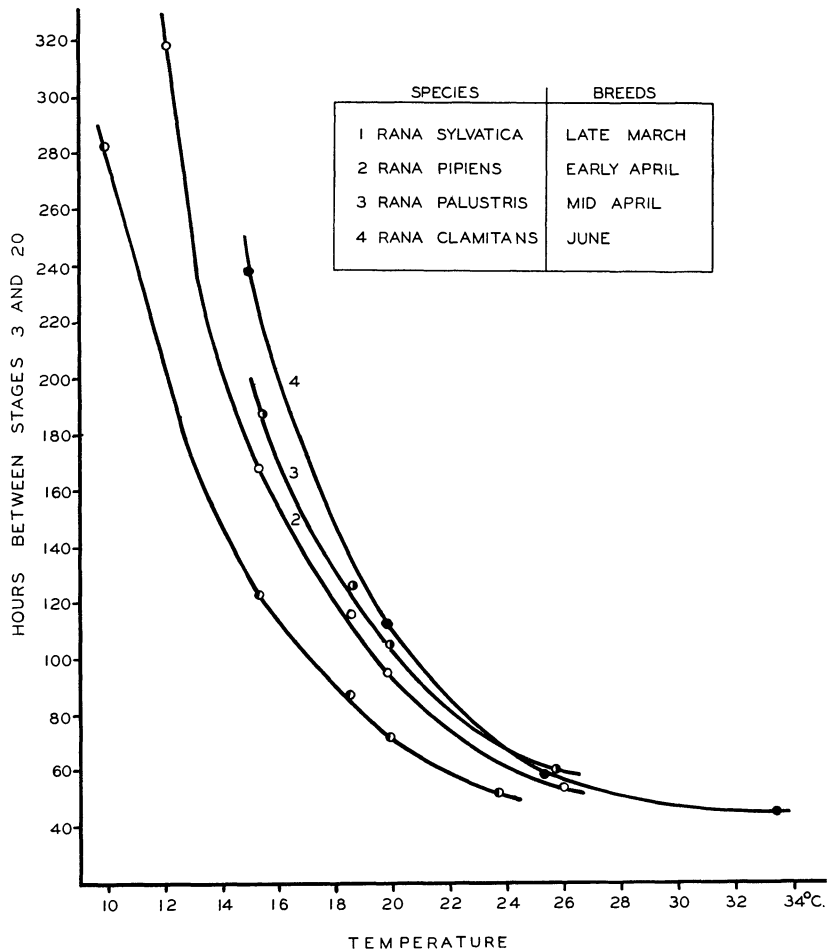


FIG. 6. A comparison of rate of development between four species of frogs. The curves represent the time to reach stage 20 at several temperatures.

is not surprising. Quite regularly also, animals that can withstand low temperatures, but cannot endure high temperatures, are more northern in their distribution than those with higher maximal and minimal embryonic temperatures. The significance of the rapid rate of development of northern animals however is not so apparent. Therefore, preliminary experiments have been carried out on two additional genera of Amphibia to learn if the rapid rate of cold-adapted species is of common occurrence in this group.

Bufo. *Bufo americanus* breeds in April in the New York City region. It ranges far to the north, having been reported from the Labrador Peninsula (Speck, '25), and throughout much of Canada. The Canadian form has been separated as *B. americanus copei* Yarrow and Henshaw by some authors.

TABLE VI. *Comparison of breeding and development of various species of Rana*

	<i>R. sylvatica</i>	<i>R. pipiens</i>	<i>R. palustris</i>	<i>R. clamitans</i>	<i>R. catesbeiana</i>
Order of breeding	1	2	3	4	5
Water temperature at time of breeding	10°	12°	15°	25°	
Most northern record	67°30'N.	60°N.	51-55°N.	50°N.	47°N.
Lower limiting embryonic temperature	2.5°	6°	7°	11°	
Upper limiting embryonic temperature	24°	28°	30°	35°	
Time between stages 3 and 20 at 18.5° C.	87	116	126	138	170

B. fowleri breeds in May in the New York City region, and extends only to southern Canada. Wright and Wright ('33) have described this as an Upper Austral species, which in a given locality breeds later than the Transitional and Canadian Zone form, *B. americanus*.

The rate of development of these forms is given in Table V. It will be seen that the data are not extensive but are sufficient to show a difference in rate. The experiment on *B. americanus* was begun when the eggs had just entered stage 7. The time between stages 3 and 7 at this temperature could not have been more than 5 hours. This would mean that *B. fowleri* requires about 107 hours from first cleavage to gill circulation (stage 20), and *B. americanus* 90 hours or less. Here again we find that a species which breeds earlier than another of the same genus, and which is distributed farther to the north, has a more rapid rate of embryonic growth.

Ambystoma. The limits of distribution of *Ambystoma* are even more imperfectly known than in the case of the frogs and toads. *A. jeffersonianum* seems to be the characteristically northern species. Cope ('89) records it from James Bay. *A. maculatum* has not been taken this far north but it does occur throughout south-eastern Canada. *A. tigrinum* reaches Canada only in the central region. *A. opacum* does not extend north of New England. Both *A. tigrinum* and *A. jeffersonianum* lay before *A. maculatum*. As they rarely occur under similar conditions it is not possible to say which of these two is the earlier breeder. *A. maculatum* breeds during the last half of March in the New York City region. *A. opacum* breeds in late September and early October in the same region. The temperature of ponds where *A. maculatum* lays has varied from 6° to 12°. Presumably it is somewhat lower when *A. tigrinum* and *A. jeffersonianum* spawn as they do so earlier in the season. The mud nests of *A. opacum* have varied from 16° to 18° when the eggs are first laid.

The observations on rate of development are given in Table VII. The

eggs of *A. tigrinum* are from two sources, those collected on Long Island, and others from the neotenic axolotl kept in laboratory tanks. Observations on those collected under natural conditions were not begun until the end of gastrulation. As the rate from this time on is indistinguishable from that of the axolotl, it is thought legitimate to use the more extensive data of the latter as representative of the species. Observations on *A. jeffersonianum* were begun when the eggs were just beginning to gastrulate. They pass through subsequent stages more rapidly than do any other species examined. This is of interest when we recall it to be the typically northern member of its genus. *A. tigrinum* develops somewhat more slowly. *A. maculatum* develops still more slowly, and *A. opacum* which lays its eggs under the warmest conditions has the slowest rate of development of any.

In figure 7 the rates of development are compared. The data for the axolotl are used as a base line. Twenty-four hours have been added to the times for *A. jeffersonianum* so that they may be compared with the other species. This amount represents the time required by *A. tigrinum* to reach stage 10 and is a conservative estimate, as in subsequent stages *A. jeffersonianum* develops more rapidly. The scatter of the points is due in part to the difficulty of referring embryos of all species to Harrison's stages made especially for *A. maculatum*.

TABLE VII. Hours of development of the eggs of various members of the genus *Ambystoma* as noted in Harrison's stages

	<i>A. macu- latum</i>	<i>A. jeffer- sonianum</i>	<i>A. opacum</i>	<i>A. tigrinum (axolotl)</i>	<i>A. tigrinum</i>
Experiments	3	1	1	3	1
Temperature	19.9±0.1°	19.9±0.1°	19.9±0.1°	19.9±0.1°	19.9±0.1°
Stage 2	0		0	0	
7	14			10	
8	16		20	14-22	
9	31-36				
10	42	0	40	24-25	
11	49.5		50	34	
12	53-55.5	4-9	60		
13	63-64	11	80	46-55	0-10
14	72-74	21			13
15	77.5	24	85	58	
16		26			
17	82				
19	88	28			
21		31	110	65-68	
23				69-72	23
24			130	76	
26	122	47	140	83	37
29				87	48
30				94	
31	146				
Heart beat	168	80		120-127	82
Gill circulation		99		146-153	106

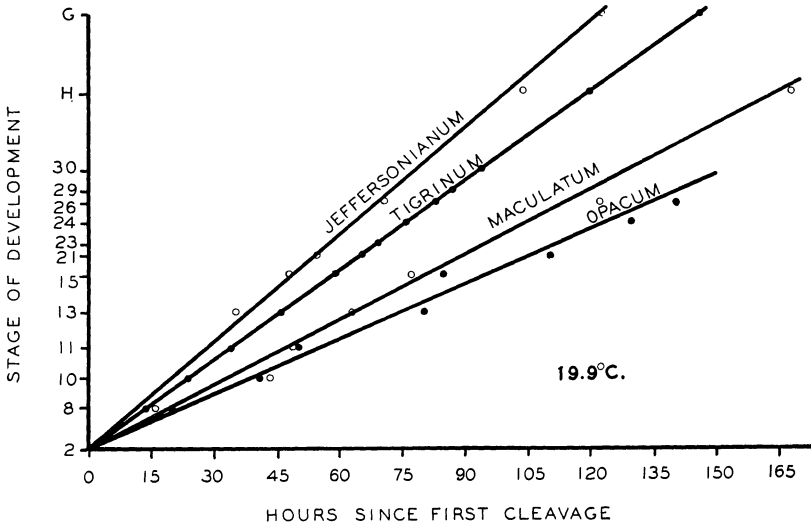


FIG. 7. A comparison of rate of development between four members of the genus *Ambystoma* at a common temperature.

The correlation between breeding conditions and rate of development shown to exist in the genus *Rana* likewise holds in these salamanders. Those species which lay their eggs early in the season when it is cold, and are more northern in their distribution, develop more rapidly than those which spawn later, and which may be considered southern species.

DISCUSSION

In previous investigations it has been noticed that variations in temperature tolerance of the eggs of different amphibians are correlated with the environmental temperature at the time of breeding, though these observations are fragmentary. Lillie and Knowlton ('97) found *Ambystoma tigrinum* more resistant to cold than *Rana pipiens*, as was pointed out previously. Hertwig ('98) found 24° to be the upper limiting temperature for the early breeding *R. temporaria*, whereas in the case of *R. esculenta* which breeds in May and June, it is 32–33°. Morgan ('02) noticed that the eggs of *R. sylvatica* were not affected by a sojourn in his ice cabinet, while those of the later breeding *R. palustris* were injured. Miss King wrote ('03), "it may be possible to show, after more species have been studied, that the maximum temperature which eggs of amphibians can endure without injury and also the temperature most favorable for their development depend, to a certain extent at least, on the time of the year at which the eggs are deposited." As can be seen in figure 5 the convictions of these earlier investigators have been substantiated.

This relationship of course is not surprising and it has been noticed in

many animal groups (Runnström, '28). It would be difficult to conceive of a species flourishing in a region where the existing temperature is lethal to any stage in its life history, unless its status is maintained by immigration from more favorable localities.

It seems, therefore, that each species of frog is best adapted to a particular geographic range, and to a special breeding season, in which conditions are suitable for development of the eggs. It is not a case of all the frogs studied being able to tolerate temperatures within a wide limit during early development, but each species is able to develop normally over a smaller range of temperatures covering conditions during the time of breeding. Hörstadius ('25) has even shown that eggs of the same species may have different temperature limits when laid at different times during the year. Thus the eggs of the sea urchin *Paracentrotus lividus* develop normally from 8° to 23° in winter when ocean temperatures are 13°, but during the summer months when the temperature has risen to 26°, the lower temperature limit is 16°, and the upper limit is 29°.

It is of interest to note that marine animals are not able to withstand as wide a range (difference between the upper and lower limiting temperatures) of temperatures as the four species of *Rana*. *Paracentrotus lividus* is able to develop normally over a range of 16° in the winter and 14° in the summer (Hörstadius, '25). Runnström ('27) examined the eggs of twelve species belonging to several phyla and found the greatest range to be 16°, and the smallest range over which normal development occurred to be 12°. On the other hand, all of the frogs are able to develop over a range of 22–24°. This greater range for frogs is doubtless correlated with the eurythermal condition of a fresh water environment, in contrast to the more constant temperature of the ocean.

The relation between temperature tolerance and rate of development has been discussed by Zawadowsky and Sidorov ('28). A survey of the literature showed them that some species could be arranged according to the velocity of cleavage in the egg. Those with the most rapid segmentation had a lower temperature tolerance and a lower Q_{10} , a relation similar to that shown to exist in frogs. To test this concept they studied the temperature tolerance and rate of development in *Ascaris megalcephala*, *A. suilla*, and *Toxascaris limbata*. They did not find support for their original hypothesis. However, their material was not especially satisfactory, and the difference in temperature tolerance among these parasitic species is very slight.

In the investigations on Amphibia the relation between temperature tolerance (and breeding time) and rate of development is too regular to be explained on a chance variation basis. Species adapted to northern conditions consistently have a more rapid rate of development than southern forms. Low temperature, however, is not the only environmental factor correlated with rapid growth. Amphibians that lay their eggs in temporary pools and

puddles must of necessity have short larval periods if they are to transform before these bodies of water disappear (Ju-Chi Li, '34; Trowbridge and Trowbridge, '37). Also amphibians found in semi-arid regions have a more rapid rate of development, according to Storer ('25). In eastern North America, however, there is an abundance of water in the spring and the differences in rate of development seem best correlated with temperature. As in the case of forms breeding in temporary bodies of water, and in semi-arid regions, the temperature effect is perhaps an adaptation to the length of time breeding sites are available, especially in the northern portion of the species' range. A form like *Rana sylvatica*, for example, can breed in March in the latitude of New York as the ponds are then free of ice, but at increased latitudes the time of the spring thaw becomes later and the winter freeze comes earlier, with the result that ponds are open for a shorter period during which embryonic growth can take place. It is thus conceivable that forms having a long larval period are better adapted to more temperate conditions. This has been shown by Zschokke ('00), who found a correlation between the length of larval life and the altitudinal limit of amphibians in the Alps. Here an increase in altitude results in a reduction of the growing season as does an increase in latitude. *Rana temporaria* with a larval period of 85–95 days reaches an altitude of 2500–2600 meters. *Hyla viridis* with a larval period of 80–98 days reaches 2200 m. *Bufo vulgaris* ranges to 2000–2100 meters. Its larval period consumes 110–120 days. *Alytes obstetricans* and *Bombina bombina* with larval periods of 120–130 and 124–134 days respectively, reach 1500–1650 and 1200–1500 meters. It would seem therefore that amphibians with long larval periods are not characteristic of regions where the ponds are free of ice for but a short time.

There are a number of cases in the literature, some of which will be cited, that indicate northern, or cold adapted species, have a more rapid rate of development at the temperatures of their environment than southern forms at these same low temperatures. Hertwig ('98) found *Rana temporaria* to develop more rapidly than *R. esculenta*. The former has a lower range of temperature tolerance and extends farther north. Races of *Lymantria dispar* from the northern part of Europe and northern Japan develop more rapidly than races from southern Europe and Turkestan (Goldschmidt, '33). Ide ('35) has shown very clearly that those species of *Ephemeroptera* characteristic of cold waters grow more rapidly under natural conditions than those found in a warmer environment. Loeb ('16) found segmentation to be more rapid in *Strongylocentrotus* than in *Arbacia* at low temperatures. The former is a characteristic species north of Cape Cod, whereas *Arbacia* is the common sea-urchin south of the Cape. Geisthardt ('37) finds the European *Cimex lectularius* to have a shorter larval period than the African *Cimex rotundatus*. The optimum temperature for development of *Cimex lectularius* is 27°, and for *C. rotundatus*, 29°. Fox ('39), who is especially interested in this prob-

lem, found the rate of segmentation in the northern *Psammechinus miliaris* to be greater than in the Mediterranean *Psammechinus microtuberculatus*.

It is suggested, other factors being constant, that animals characteristic of northern, or cold regions, have a greater growth rate at the temperatures of their environment than closely related southern forms at these same low temperatures. Orton ('23) has expressed a similar view, thus he states, "it does not necessarily follow that because the rate of metabolism in tropical or temperate animals falls off rapidly with decreasing temperatures approaching 0° C., that metabolism in polar animals is necessarily of the slow rate of temperate animals at polar sea-temperature. No reason has yet been shown that adaptation of metabolism cannot occur; on the contrary there is every reason to expect such adaptation." Whether this correlation is as universal as the other rules expressing regularities in geographical variations (Dobzhansky, '37) remains to be seen.

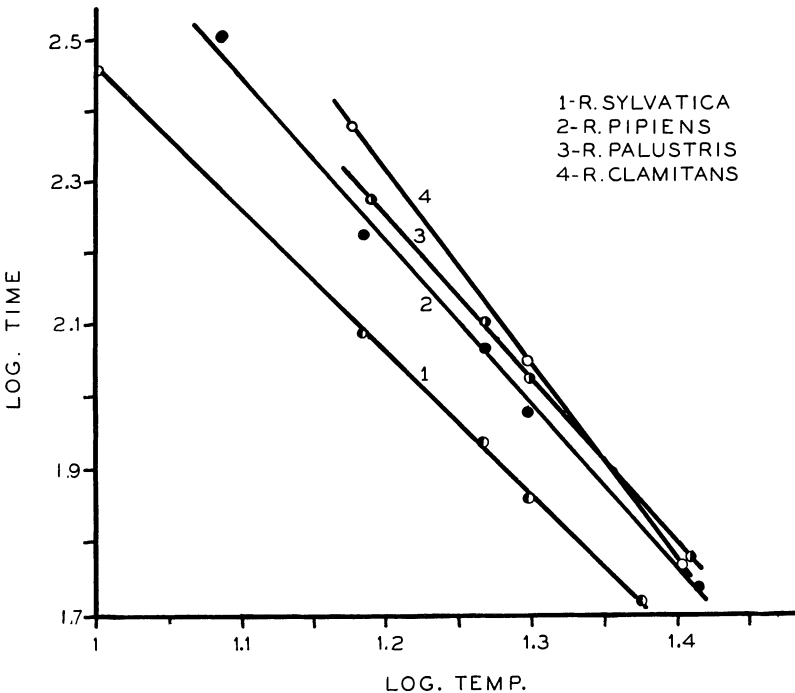


FIG. 8. A log. plot of the data in figure 6.

We have pointed out previously that the differences in rate of development are greater at low temperatures. This becomes more apparent when the data on time to reach stage 20 (Fig. 6) are plotted as logarithms (Fig. 8). It is then seen that not only is the entire curve shifted to the right with an increase in temperature tolerance, but the slope is greater. This increase in

the slope of the curve may even result in a slightly greater rate of development. Thus at temperatures above 25° *Rana clamitans* reaches stage 20 in less time than *R. palustris*, whereas at lower temperatures the latter develops much more rapidly. Loeb ('16) noticed this in a comparison of segmentation rates in *Arbacia* and *Strongylocentrotus*. At low temperatures the northern *Strongylocentrotus* develops more rapidly, but at temperatures above 15° this order is reversed. Bělehrádek ('35) has discussed the relation between temperature tolerance of eggs and his temperature coefficient b (value for the slope of the line representing the time to reach a given morphological stage at a number of temperatures in a log-log plot). The data given in this paper confirm his hypothesis that "the temperature coefficient of analogous biological processes increase with the adaptation of the protoplasm to higher temperature." The value of b for *Rana sylvatica* with an upper limiting temperature of 24° is 2.0. For both *R. pipiens* with an upper limiting temperature of 28° (but see notes under "Experimental"), and *R. palustris* with an upper limit of 30°, b is 2.3. The value of b in the case of *R. clamitans* which can tolerate temperatures as high as 35°, is 2.6.

A point of interest in the figures for development of each species (Figs. 1-4) is the apparent divergence of some of the curves at advanced stages. This increase in the value of the temperature coefficient with the stage of development reached, has been noticed by other investigators and is discussed by Bělehrádek ('35). The significance is not known.

SUMMARY

1. A correlation was found to exist between breeding habits of Amphibia, their geographical distribution, and the temperature tolerance and rates of development of the eggs (Table VI).

2. Frogs breeding when environmental temperatures are low have lower minimal and lower maximal temperatures than species breeding when pond water is warmer.

3. The range (difference between upper and lower limiting temperatures) over which normal development is possible is 22-24° in the four species of frogs studied.

4. Frogs, toads, and salamanders breeding when temperatures are low develop more rapidly than forms breeding under warmer conditions.

5. The early breeding species are in general more northern in their distribution than forms breeding later.

6. The temperature coefficient of development is lower in northern species than in southern species of frogs.

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