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The effect of temperature on the growth rates of several California frogs: a thesis ...

Donald Avila Villeneuve

University of the Pacific

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THE EFFECT OF TEMPERATURE ON THE
GROWTH RATES OF SEVERAL CALIFORNIA FROGS

A Thesis
Presented to
The Faculty of the Department of Zoology
College of the Pacific

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by
Donald Avila Villeneuve

August 1960
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The writer further wishes to acknowledge a debt of gratitude to Dr. Philip C. Dumas, under whose direction as an undergraduate the work on Rana pretiosa was done and who is responsible for the basic idea from which the temperature gradient tank used in this work was developed.

For being so generous with his time in helping to collect the many specimens used and for his assistance in a multitude of other ways the writer wishes to thank Mr. Wayland L. Ezell, College of the Pacific.

The writer is also appreciative for the assistance given by mail of Dr. John A. Moore, Columbia University, New York, Dr. Robert M. Storm, Oregon State College and Donald G. Dunlap, South Dakota State University.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>10</td>
</tr>
<tr>
<td>Temperature Tolerance and Rate of Development</td>
<td>15</td>
</tr>
<tr>
<td>Rana aurora draytonii</td>
<td>15</td>
</tr>
<tr>
<td>Rana aurora boylei</td>
<td>19</td>
</tr>
<tr>
<td>Rana catesbeiana</td>
<td>23</td>
</tr>
<tr>
<td>Discussion</td>
<td>27</td>
</tr>
<tr>
<td>Summary</td>
<td>33</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>35</td>
</tr>
<tr>
<td>Explanation of Plates</td>
<td>38</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Comparison of Times to Stage 20 at Various Temperatures of <em>R. catesbeiana</em> Embryos from Louisiana and California</td>
<td>23</td>
</tr>
<tr>
<td>II. The Relation Between Geographical Distribution, Onset of Breeding, and Adaptive Embryological Characteristics</td>
<td>27</td>
</tr>
<tr>
<td>III. A Comparison of the Upper and Lower Limiting Embryonic Temperatures of Several Eastern Ranids</td>
<td>31</td>
</tr>
</tbody>
</table>
INTRODUCTION

The study of the effect of temperature upon living systems has been of interest to biologists since the earliest emergence of the theoretical phase of the science. It was found that some of the most profound effects of temperature variation were manifested in the early developmental stages of the organism. The literature shows a long history of experimentation with the effects of various mechanical and chemical stimuli upon the subsequent embryonic development; amphibians in particular have been a favorite subject for this work. The first quantitative data concerning the effect of temperature upon the rate of growth in amphibian embryos was that of Lillie and Knowton, (1897). They found that as temperature was increased above the optimum for the eggs of Ambystoma tigrinum or Rana pipiens the time necessary to reach a given morphological stage became progressively less. They also recognized the relationship between the effects of temperature upon growth and the effects of temperature upon the rates of chemical reactions. They surmised then: "...
each species of frog is best adapted to a particular geographic range...breeding season, in which conditions are suitable for development of the eggs..."

This work was followed a year later in 1898 with the publication of Oscar Hertwig's work on the effect of
temperature upon the development of the two European frogs, *Rana fusca* and *Rana esculenta*.

Krogh's (1914) extensive monograph on the relationship of temperature to development was a major contribution and is considered an outstanding work on the subject. Crozier (1926) studied growth with special attention to the effect of temperature. Julian S. Huxley in 1926, and again in 1927 with M. A. Tazelaar, investigated the results of modification of growth rates by varying the temperature under which frog and chick embryos were raised. F.G. Gilchrist (1928) studied the effect of temperature upon the development of the urodele, *Triturus torosus*.

In 1935, a paper by Meyer Atlas provided an excellent, detailed, highly quantitative report on the relationship of temperature to development in *Rana pipiens*. Since there were no generally accepted tables for the development of amphibian larvae Atlas chose several stages of morphological development as reference points. These stages correspond to the following stages of Shumway (1940):

<table>
<thead>
<tr>
<th>Atlas' stages of development</th>
<th>Shumway (1940) stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) The first cleavage stage</td>
<td>3</td>
</tr>
<tr>
<td>2) Invagination of the ventral lip (fusion of lateral lips)</td>
<td>Midway between 11 and 12</td>
</tr>
<tr>
<td>3) Fusion of Neural folds</td>
<td>15</td>
</tr>
<tr>
<td>4) Growth stages</td>
<td>20-23</td>
</tr>
<tr>
<td>5) Completion of opercular fold</td>
<td>25</td>
</tr>
</tbody>
</table>
It was found that at a constant temperature the number of cells being produced at any time is proportional to the number of cells present at that time. This relationship is similar to the "law of mass action" of chemical reactions. In the quantitative analysis of the effect of temperature on development, Atlas found that the equation expressing this relationship reduces to the Arrhenius equation so widely used in theoretical analysis of velocities of chemical reactions. In summarizing this work Atlas reported that: (1) There is a progressive increase in tolerance of elevated temperatures with size of embryos, (2) The cleavage rate is constant to the 64-cell stage (Shumway stage 8). (Beyond this stage internal development and decreased cell division alters rate of cleavage.) (3) Relation of gill growth to body growth is altered by temperature in such a way that the relative size of the external gills decreases as temperature decreases. (4) The effects of temperature upon the growth of embryos is identical for different stages of development in the lower and intermediate temperature ranges, but the rate of growth diverges at higher temperatures indicating separate limits of tolerance at different stages.

In this same year, 1935, Belehradek published his monumental work on the subject of temperature and its
effects upon the living system, in which the knowledge
to that date was summarized. This work remains one of
the most widely used references upon this subject.

Up until this time workers were handicapped by not
having an accepted series of morphological end-points in
the developing amphibian larva, by which their data would
be placed on common ground for comparative purposes. This
was facilitated with the publication, in 1937, of Pollister
and Moore's paper on the stages in the development of Rana
sylvatica, which was followed in 1940 by the widely used
Shumway tables of development of R. pipiens.

Ryan (1941) published his results of the effect upon
the subsequent rate of development of Rana pipiens embryos
after exposure to various temperatures. In his work, Ryan
submitted developing embryos to an experimental temperature
then removed them to the control temperature and compared
the subsequent growth rate with that of embryos that had
been kept at the control temperature. It was found that
the rate of cleavage and later development in R. pipiens
can be influenced by previous temperature history. Removal
to a higher temperature after exposure to a low temperature
results in an acceleration of growth that will, for a time,
exceed that of controls kept at a constant higher temperature.
The reverse is true when the embryos are kept at a high temperature and then moved to a low temperature. Acclimatization after increase or decrease of temperature with eventual return to the normal developmental rate was also demonstrated. Ryan postulated the possibility that these modifications in rate of development are the result of two different mechanisms, one operating at high temperatures and the other operating at low temperatures, the actions of which results in a break in the curve of the rate of development with temperature.

Another work published in 1941 was that of G. P. DuShane and C. Hutchinson. Their work was concerned with the effect of temperature upon the form and the behavior of developing embryos. Their results showed that form and behavior are affected by temperature differently. The development of behavior is more strongly inhibited by low temperature than is the development of form.

Even the earliest workers in this field recognized the inherent ecological importance of the effect of temperature upon growth rate, but for the most part their work was concerned primarily with the physiological results. The work of J. A. Moore (1939, 1942a, 1942b) introduced a new approach to this problem: the application of experimental laboratory data to ecological problems such as distribution, range
limitations, breeding habits and, lately (1949, 1957), speciation and evolution. Moore has demonstrated several consistent correlations between the laboratory-determined embryonic response to various temperatures and the aforementioned ecological problems. These correlations are here stated:

1) A relationship exists between breeding habits of Amphibia, their geographical distribution, and the temperature tolerance and rates of development of eggs.

2) Frogs, breeding when environmental temperatures are low, have a lower minimal and maximal tolerance.

3) Frogs, toads and salamanders, breeding when temperatures are low, develop more rapidly than warmer water species at low temperatures.

4) Early breeding species generally have a more northerly distribution.

5) The temperature coefficient ($Q_{10}$) of development is lower in northern species than in southern species of frogs. (Moore, 1939).

Care must be used in the application of these tentative principles. Moore's work was with frogs distributed in an area in which there is usually sufficient water to sustain amphibians. Moore has cautioned that temperature alone is not the only factor selecting for rapid development.
Amphibians occupying an area in which rainfall is scarce or intermittent also must develop rapidly before the water disappears. In an area such as California, with definite seasonal rainy periods and prolonged periods of little or no rainfall, a rapid embryonic development may indicate an adaptation to this cycle rather than to temperature.

To date the majority of the work on the ecological significance of embryonic temperature relationships has been with Eastern frogs. The writer is aware of only two papers describing work of this nature with California frogs: Schechtmann and Oleson (1941) working with temperature tolerance in embryos of *Hyla regilla*, and the work of Zweifel (1955) on the *Rana boyleri* species group.

For this reason it seemed desirable to study the effect of temperature on the growth rates of some frogs native to California and, since work of this nature has been done with the eastern *Rana catesbeiana* by Moore (1942b), it afforded an excellent opportunity for comparison to repeat the study using the California *R. catesbeiana*.

Although the California bull-frog has a considerable distribution, it is not native to this state but was introduced around 1905 (Storer, 1925). Very little work has been done with the life history of this species despite the opportunity it would seem to afford biologists interested
in adaptational aspects of ecology. Storer (1925) stated that, from the data available at the time, the seasonal schedule with regard to time of emergence from hibernation, time of spawning, and length of time required for larval development, conformed closely to its calendar in the eastern states and had not, to that time, been modified to local conditions.

The relationship of the two native California frogs, *Rana aurora draytonii* and *Rana boylei boylei*, is one of close habitat partition. *R. aurora* is a pond frog and prefers permanent and relatively still water. *R. boylei* is primarily a stream frog and is found mostly along relatively small, gravelly watercourses. Although they seem to demonstrate a definite habitat preference some workers have observed these frogs in apparently sympatric relationship. (Stebbins 1951; Storer, 1925; Zweifel, 1955).

By application of the inter-relationships as proposed by Moore, one could expect to find *Rana aurora* to have a more northerly distribution, a larger diameter egg, lower minimal and maximal temperature tolerance, lower temperature coefficient of development, and a higher developmental rate than the *R. boylei*. *Rana aurora* does in fact range much farther northward than *R. boylei*, which occurs only in California and in Southern Oregon. (Although the subspecies,
R. aurora draytonii, dealt with here occurs almost exclusively in California. (Stebbins, 1951). The diameter of the egg deviates from the prediction slightly. The subspecies of R. aurora that occurs in California (Rana aurora draytonii) has almost the same sized egg, 2.06 – 2.50 mm. (Storer, 1925) as that of R. boylei, 2.2 mm. (Stebbins, 1951). If the distribution of the entire species is considered, the correlation is valid. The most northerly distributed R. aurora aurora has an egg 3.04 mm, in diameter. (Ibid).

The present experiment was designed to test the remaining predictions.
METHODS AND MATERIALS

Most of the frogs used in this experiment were collected just prior to or during their respective breeding periods.

The majority of the *Rana boylei* were collected along the banks of Sutter Creek, Amador County, approximately three miles west of the town of Volcano at about 2000 feet elevation. This creek represents an ideal *R. boylei* habitat and is located in the digger pine-interior oak, Lower Transition zone of the Sierra. The flow in this stream is continuous all year and runs for the most part in a fairly deep canyon at a moderate gradient. Some *Rana boylei* were also collected from a stream at the foot of the east slope of Mt. Diablo, Contra Costa county. The flow in this stream is moderate during the spring but it diminishes considerably during the summer, although many deeper pools undoubtedly retain a quantity of water. The frog specimens in this stream were, on the average, of a larger size than the Sutter Creek specimens and differed from the Sutter Creek specimens in coloration. Zweifel (1951) found no difference genetically between separate races of these frogs despite color differences.

Although a considerable amount of time was spent attempting to catch pre-breeding condition *Rana aurora*,
II.

The only frogs of this species that were captured were three females and one male from a deep, heavily shaded pool lying along the main course of Sutter Creek. These individuals were caught on April 9, 1960 with the water temperature at 14° Centigrade. An egg mass containing embryos in a late pre-hatching condition was also observed in the pool at this time. It is interesting to note that several R. boylei were captured in this same pond at this time. The data used in this paper for R. aurora were obtained from an egg mass which was collected on February 3, 1960 in a large pond near Pine Grove, Amador County at 2200 feet elevation. At the time of collection the air temperature was 7° Centigrade and the water was 9° Centigrade. The eggs were collected in the two-celled stage of development and were carried back to the laboratory where they were separated and placed in large crystallization dishes at various temperatures. The stage of development, at the time of separation, was the fifth cleavage stage, (Shumway, 1940). The observations of this group of eggs were not as well controlled as in later experiments since temperature control equipment was not yet available. The data are included here primarily for comparison with the data obtained for the other two species. It
is hoped that a more controlled experiment with R. aurora can be made at a later date.

Eggs used in the experiment, with the exception of the afore-mentioned Rana aurora were obtained by the subperitoneal injection of anuran anterior pituitary into mature, gravid females using the method described by Rugh (1948). These eggs then were stripped into a sperm suspension prepared by the maceration of two pairs of testes in approximately 20-30 cc. of pond water which was spread in a thin film over the bottom of a large crystallization dish. The eggs were allowed to stand in the sperm suspension for 15 minutes, at which time they were flooded with fresh pond water. After 20 minutes the water was drained off and the eggs again were flooded with water. After one hour those eggs that had rotated so that the animal hemisphere was uppermost were assumed to be fertilized. At the onset of the first cleavage stage the egg mass then was cut into small clusters and 50 eggs were transferred to each of seven large dishes that were at previously established temperatures. (See Plates 3, 4). The eggs then were observed at frequent intervals for the first 24 hours and at precisely every 24 hours thereafter. The development stage reached by 50% of the embryos was recorded.
The temperatures below 10° Centigrade were maintained by refrigeration with a probable temperature variation of less than one degree. The remaining temperature gradients were maintained in a gradient tank constructed by the writer from a design suggested by Dr. Philip Dumas of the University of Idaho, (See Plates 1 and 2). This tank consisted of a wooden frame of 1/2 inch thick plywood, six feet by three feet by one foot deep. The inside of the tank was covered with fiber-glass and was partitioned laterally into five compartments, each fourteen inches wide, by aluminum dividers three inches high. The water level was maintained to just below the tops of these dividers. The dishes containing the embryos were elevated one inch above the bottom of the tank and arranged in rows of three abreast, within each of the five compartments.

The cool end of the tank was maintained by a large capacity, one-quarter Horse Power compressor through immersed copper coils. The temperature at this end was regulated by a ±1° thermo-regulator.

The high-temperature end of the gradient tank was warmed by heating a low viscosity, high temperature, anhydrous oil (Shell, Clavus oil) in a controlled temperature
container. The hot oil then was circulated through copper coils immersed in the gradient tank. By the proper manipulation of the thermo-regulator that controlled the compressor and the degree of heat of the oil being circulated, a variety of temperatures were established with a degree of homogeneity that would be difficult to attain by using individual water baths or ovens. Over the period of this investigation, it was found that this apparatus demonstrated a total variation of less than one degree.

The method used to maintain the temperatures for the *Rana aurora* experiment was similar to the above procedure with the exception of the series maintained in the gradient tank which was not yet completed. For the high and mid-range temperatures, a combination of temperature controlled rooms and an oven were used. A temperature of 35° Centigrade was maintained in a paraffin warming oven which was thermostatically controlled to ±1° Centigrade. The 22.5° Centigrade, and 25.5° Centigrade groups were maintained in separate rooms in which the temperatures were kept fairly constant with an average fluctuation of less than 5° Centigrade. The group kept at 11° Centigrade were rather well-controlled in a water bath cooler which had a ±1° Centigrade differential.
TEMPERATURE TOLERANCE AND RATE OF DEVELOPMENT

1. Rana aurora draytonii. As stated above, the R. aurora eggs used in this experiment were obtained in the field and were in the fifth cleavage stage before they were transferred into separate temperatures. The data is further prejudiced by inadequate control of some of the experimental temperatures; particularly those of the intermediate temperatures. Despite these considerations the writer feels these data are sufficiently accurate to justify their comparison with the data obtained under better controlled conditions in later experiments.

One group of the eggs was placed in an oven at 35° (all temperatures are in degrees centigrade); two more groups were placed in separate rooms that had average daytime temperatures of 25.5° and 22.5° respectively; the remaining temperatures of 11°, 8.5°, and 6° were maintained by refrigeration. All of these temperatures, except the 25.5° and 22.5° were thermostatically controlled. The two rooms were maintained at a fairly constant daytime temperature, but the nighttime temperature dropped for a period of about six hours each night. For this reason the growth curves obtained for these two temperatures (Plate 3, Figure 1) are probably skewed somewhat to the right.

35° C. Embryos placed at this temperature died shortly after being exposed. The jelly capsules became
highly vacuolated and the eggs appeared to disintegrate.

25.5°C: The eggs developed quite rapidly at this temperature. Stage 8 was reached in a little less than five hours, stage 10 in 20 hours, stage 17 in about 75 hours and hatching in slightly more than 100 hours. Although some of the embryos developed normally, many exogastrulated or developed protruding yolk plugs, and only 50 per cent of the embryos successfully reached stage 20. The upper temperature limit for embryonic development of *Rana aurora* would then appear to lie somewhere between 22.5°C and 25.5°C. Since embryonic development at 25.5°C was partially successful it would seem that this temperature is fairly close to the upper limit. Later experiments demonstrated that there is a point approaching either the upper or lower limits of embryonic temperature tolerance at which embryonic development is inhibited and mortality increases. Apparently there are variations in the temperature tolerances of individuals of sibling groups which become apparent as the environment becomes more rigorous.

22.5°C: Development at this temperature proceeded normally although somewhat slower than the previous group. Stage 8 was reached at seven hours, stage 10 at about 30 hours, stage 17 at about 120 hours and hatching between 150 and 174 hours. It should be noted here a relationship between this temperature and the previous temperature that later proved to be characteristic. As temperature decreased
the time for the embryos to reach a specific stage of development increased so that the lines describing these growth rates as drawn on a graph (Plates 3 and 4) increasingly diverge from a common starting point. This relationship has previously been described by Meyer Atlas (1935). The percentage of normal tadpoles hatching at this temperature was considerably higher (87 per cent) than at 25.5°.

11.0° C. Stage 8 was reached in about 17 hours, which was 7 hours more than the 22.5° group took to reach this corresponding stage of development. From stage 8 on the time lag between the two groups became increasingly more apparent. Stage 10 required 60 hours, stage 15 was reached after 225 hours and hatching occurred at almost 320 hours—about 265 hours longer than the time needed for development at 22.5°.

8.5° C. The rate of development in the early stages at this temperature was very close to the rate of development in the early stages of the 11° group and fairly normal except for some pigment scattering. At stage 10 (formation of the dorsal lip) the invagination of cells appearing impeded and most of the embryos failed to gastrulate properly, developing large, incomplete protruding yolk plugs. Development of those that succeeded in gastrulation properly proceeded slowly with stage 12 being reached in
160 hours. At this stage of development many apparently successful gastrulae began to develop a curious "ring" shape which has been attributed by Rugh (1948, p. 76) to the anterior end of the future embryo being pulled inward as the result of impeded invagination. None of the embryos kept at this temperature had formed a neural plate at the time this experiment was terminated, although several reached stage 14 by 320 hours. The few surviving embryos all had formed "rings" or some other abnormality, such as a protruding yolk plug or highly scattered pigment, indicating that this temperature was below the minimal temperature for development of this species. It is interesting to note that the Rana aurora embryos failed to develop properly in the laboratory at a constant 8.5° temperature, particularly since this egg mass was obtained from a pond having an early morning water temperature of 9°.

The embryos kept at 6°. At this temperature development was very obviously inhibited from the start. Stage 8 was reached after more than 50 hours, but the vitelline membrane had taken on a bluish tinge and in many of the embryos severe depigmentation was evident. After about 130 hours about 20 percent of the embryos had reached stage 10, although the jelly surrounding them had a cloudy appearance.
None of the embryos developed beyond stage 12 with all of the gastrulae formed being abnormal. In these embryos, and embryos subjected to subminimal temperatures in later experiments, the inhibition of proper gastrulation appeared general. This apparent difficulty resulted in a characteristic uneven development of blastopore, protruding yolk plugs or exogastrulation. Those embryos that managed to more or less successfully develop gastrulae invariably developed the characteristic ring form.

2. *Rana boylei boylei*. Eggs used in this experiment were obtained by the induced ovulation method previously described. Although transfer of the embryos was begun at stage 3, over 50 percent of the embryos were in early stage 4 by the time transfer was completed. Temperatures used were obtained by use of the temperature gradient tank described above (Plate 1 and 2), except for 7°, which was obtained by refrigeration.

32°: Development at this temperature ceased shortly after transfer of the embryos to the gradient tank. Severe cytolysis and the characteristic air bubbles in the jelly capsules were general.

27°: At this temperature development was very rapid, with stage 6 being reached in less than 20 hours. Dorsal lip formation was first evident in about 30 hours and was
apparently normal. Although the early stages of gastrulation appeared normal, over 50 percent of the embryos failed to gastrulate properly at this temperature with protruding yolk plugs very prevalent. A curious dorsal-lateral flattening occurred in many of the gastrulae during the neural plate stage (Stage 14) at approximately 50 hours. Stage 17 was reached in 75 hours and hatching occurred at 125 hours. Only 10 percent of the embryos raised at this temperature survived to stage 20 and all but six of these appeared abnormal. It would appear from the above results that 27°C is very close to the upper limiting temperature for normal development of R. boylei embryos.

22°C. Embryos at this temperature developed quite normally. Stage 8 was reached in a little less than 20 hours, gastrulation was completed in a little over 50 hours, tail bud formation began in less than 100 hours and stage 20 was reached by 150 hours with over 90 percent of the embryos hatching normally.

15°C. Development of the embryos placed at this temperature began to deviate considerably from the higher temperature groups of embryos after stage 8 was reached in about 25 hours. Although the embryos kept at 15°C required only 5 hours more time to reach stage 8 than the
embryos kept at 22°, the time required for the 15° group to reach stage 10 was over 20 hours more than the time required for the 22° group to reach stage 10, and nearly 165 hours separated the hatching of the two groups. It is possible that this relative similarity in early growth rate, with gradual divergence as development increases, may partially be due to the fact that early development through the first cleavage stage of all the embryos was completed before the separation into the experimental temperatures, and therefore was, for awhile, at one temperature. There may be a time lag before the new temperatures begin to exert their effects upon the metabolism of the developing embryos.

Development of the embryos at 15° was slow but normal through stage 20 with over 95 percent hatching. The times to reach the different stages were as follows:

Stage 8: 25 hours, stage 10: between 55 and 60 hours, stage 12: 100 hours, stage 14: 145 hours, stage 15: 175 hours, stage 17: 220 hours and stage 20: 320 hours.

10° C. The development of embryos at this temperature was extremely slow and appeared to be just barely subminimal since a few of the embryos had reached stage 18 after over 500 hours. Since the rate of development was so
retarded, with less than 10 percent of the embryos surviving to this late stage, it could be assumed that development at this temperature in nature would be prohibited. Stage 8 was reached in about 50 hours with no apparent abnormalities; stage 10 was not reached until almost 70 hours later, 120 hours after stage 4. Development continued slowly, stage 12 was reached 200 hours after stage 4. Gastrulation was accomplished by a little over 50 percent of the embryos, the remaining embryos showing the usual symptoms of impeded invagination. Neuralae were formed by those embryos succeeding in gastrulating properly and rotation took place at approximately 30 hours. These embryos were kept under observation for nearly 600 hours, but after they had reached stage 18 (500 hours) development appeared to cease. Only 20 percent of the embryos survived to stage 18, and 100 hours later when the experiment was discontinued only 10 percent of the original total, five individuals, appeared to be alive. All of the embryos during the later stages (17-18 or 19) showed a severe lateral flexure of the posterior one-half of the body as if cramped into a too-small space. It appeared as if at cold temperatures the jelly membrane surrounding the developing embryos failed to expand as the embryos grew.
7° C. All of the embryos raised at this temperature failed to gastrulate properly. Stage 8 was reached in about 75 hours at which time abnormalities such as unequal cleavages, depigmentation and cytolysis began to appear. Abnormalities increased as stage 10 was reached between 190 and 200 hours. Stage 12 was reached by only a few of the embryos and gastrulation in these was highly abnormal all of them forming protruding yolk plugs.

3. *Rana catesbeiana*. Data for this species was obtained in the same manner as that described above for *Rana boylii*. The temperatures at which embryos of this species were raised were similar to the temperatures which J. A. Moore used in his work with *R. catesbeiana* embryos from Schriever, Louisiana in 1942. The following table represents the time required to reach stage 20 at various temperatures for *R. catesbeiana* embryos from Louisiana and California:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time to Stage 20</th>
<th>Temp. Condition</th>
<th>Stage 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schriever, La.</td>
<td>Stockton, Calif.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.3°</td>
<td>killed</td>
<td>12.0°</td>
<td>killed</td>
</tr>
<tr>
<td>14.8</td>
<td>many deaths, irregular; some survival</td>
<td>19.0</td>
<td>normal</td>
</tr>
<tr>
<td>19.8</td>
<td>normal</td>
<td>131 hours</td>
<td>30.0</td>
</tr>
<tr>
<td>21.6</td>
<td>normal</td>
<td>71 hours</td>
<td>32.0</td>
</tr>
<tr>
<td>23.9</td>
<td>normal</td>
<td>18½ hours</td>
<td>35.0</td>
</tr>
<tr>
<td>33.3</td>
<td>killed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.6</td>
<td>killed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
From the above comparison it would appear that the rate of development of the California bullfrogs is entirely comparable to the rate of development of bullfrogs from Louisiana. On the other hand, the California frogs seem to have shifted the range of embryonic temperature tolerance somewhat upward. The Louisiana frogs developed at 14.8°, although somewhat injured, leading to the conclusion that 15° would be the lower limiting temperature. The upper limit of embryonic temperature tolerance for the Louisiana frogs, according to Moore, is near 32°. The California frogs failed to develop at 16° and developed quite well at 32°. Since development near the temperature limit resulted in progressively more injury in previous experiments it would appear that the upper temperature limit for development of California R. catesbeiana embryos is above 32°, probably 33°. This would indicate a shift of about 1° for the entire developmental temperature range of the California R. catesbeiana.

The development of the Rana catesbeiana embryos at the various temperatures was as follows:

35° C. Although they reached stage 12 in a little over 12 hours none of the embryos gastrulated properly, and development proceeded no further.

32° C. Early development at this temperature was quite close to the rate of development at 35°. Unlike
the $35^\circ$ group embryos at $32^\circ$ gastrulated with less than 10 percent abnormality and continued to develop until hatching at about 36 hours.

$30^\circ$ C. Early development to stage 11 again was quite close to the previous temperature. After gastrulation started, however, the developmental rate began to diverge from that of the embryos at $32^\circ$. Gastrulation was completed between 18 and 20 hours, tail bud formation (stage 17) commenced at a little over 30 hours and stage 20 was reached at about 48 hours with approximately 90 percent of the embryos hatching normally.

$24^\circ$ C. Embryos raised at this temperature developed with a high degree of viability, with almost 100 percent hatching. Stage 8 required a little over 12 hours, stage 12 less than 26 hours, gastrulation was completed in 36 hours, stage 17 was reached in 50 hours and hatching in 74 hours.

$19^\circ$ C. Development at this temperature was considerably slower than at the previous temperatures. Stage 8 was reached in over 10 hours, stage 10 required 24 hours and gastrulation began after more than 40 hours. Completed neural plate developed after more than 60 hours and hatching took place at approximately 126 to 128 hours. About 90 percent of the embryos hatched and appeared quite normal. There was a morphological difference in
gill length between embryos raised at this temperature and those embryos raised at 32° and 30°, with the 24° group being approximately intermediate. Embryos raised at the higher temperatures developed very long, branched gills compared to the gills developed by embryos raised at the lower and intermediate temperatures. This relationship of gill length to temperature has previously been described by Meyer Atlas (1935) who observed this relationship in embryos of the frog *Rana pipiens*. This morphological adaptation to temperature was only noticed in the embryos of *Rana catesbeiana* in this present work, although it probably was present in the embryos of the other two species of frogs, although to a lesser degree.

16° C. Embryos raised at this temperature began to show abnormalities after 12 hours (stage 8) when some depigmentation in the cleavage furrows became apparent. At stage 12 (60 hours) it appeared as if gastrulation was impeded and after 82 hours observation of these embryos was discontinued since they had all failed to gastrulate properly. The lower limiting temperature for embryonic development in *Rana catesbeiana* of California is evidently somewhere between 19° and 16°.
DISCUSSION

With the above data it should be possible to compare the three species of frogs and see how well they correspond to the correlations regarding embryonic temperature tolerance, rate of development, and geographical distribution proposed by Moore. The following table lists the information necessary for establishing these relationships. The information regarding egg size, distribution, breeding period and type of jelly mass has been compiled from Stebbins (1951), Storer (1925), and Zweifel (1955).

TABLE II
The relation between geographical distribution, onset of breeding, and adaptive embryological characteristics.

<table>
<thead>
<tr>
<th></th>
<th>R. aurora draytonii</th>
<th>R. boylei</th>
<th>R. catesbeiana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern limit</td>
<td>41°</td>
<td>45°</td>
<td>50°</td>
</tr>
<tr>
<td>Southern limit</td>
<td>31°</td>
<td>33°</td>
<td>32°</td>
</tr>
<tr>
<td>Onset of breeding</td>
<td>Jan.-Mar.</td>
<td>Mar.-May</td>
<td>June</td>
</tr>
<tr>
<td>Lower limiting embryonic temperature</td>
<td>9°</td>
<td>11°</td>
<td>17°</td>
</tr>
<tr>
<td>Upper limiting embryonic temperature</td>
<td>24°</td>
<td>26°</td>
<td>33°</td>
</tr>
<tr>
<td>Hours to stage 20 at 20°C</td>
<td>175</td>
<td>195</td>
<td>120</td>
</tr>
<tr>
<td>Egg diameter in mm.</td>
<td>2.06-2.50</td>
<td>1.9-2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Type of jelly mass</td>
<td>Compact, submerged</td>
<td>Compact, Submerged</td>
<td>Film, at surface</td>
</tr>
</tbody>
</table>
It appears from this data that the two frogs *Rana aurora draytonii* and *Rana boylei* fit the correlations as described by Moore quite well, with the possible exception of the northward distribution. The figure given above for the northward distributional limit of *R. aurora* is for the subspecies *draytonii*, which occurs entirely in California. The distribution of the entire species is considerably more than in California as the subspecies *R. aurora aurora* is found as far north as Vancouver Island, British Columbia. Storer (1925, p. 244) has said: "*Rana aurora draytonii* shows less special adaptation to the climatic conditions of the Pacific Coast than many of the other species of western amphibians. It seeks and remains in situations where the water supply is permanent and therefore has no need to develop special adaptive responses to the semi-arid climate. . . . General dispersal . . . probably occurred at some time in the past when conditions were less arid." If this is true then it is quite probable that the subspecies *R. aurora draytonii* may have retained the same adaptive embryological characteristics as the more northerly distributed *R. aurora aurora* from which it appears to have been evolved. *R. aurora draytonii* also appears to have extended its range further south than the *R. boylei* despite the fact that *R. boylei* embryos are adapted to higher temperatures.
This can be explained by the fact that at the southernmost limits of its range *R. boylei* *boylei* is replaced by the similar subspecies *R. boylei muscosa*. Zweifel (1955, p. 273) has proposed that the frogs of the *boylei* species group at some time in the past had a much broader southward distribution than at present, but increasingly drier conditions in the later Tertiary probably brought more breaks between members of the group resulting in several disjunct populations which later became separate species.

Two characteristics common to *Rana boylei* and *Rana aurora*, which may show adaptation to the unique climate and rainfall of California, are the comparatively slow rate of development and the rather narrow range of embryonic temperature tolerance. Although both of the California frogs required well over 150 hours to reach stage 20 at 20°F, none of the eastern frogs studied by Moore required much more than 130 hours to reach stage 20 at 20°F. In fact most of the eastern frogs required only a little over 100 hours to reach the hatching stage. Since extreme cold is not a problem within the range of these two California ranids, the growing season is extremely long as opposed to a relatively short period of activity for the eastern amphibians. This being the case there would be no premium
placed upon rapid development in California since there is sufficient time to complete the larval period and metamorphosis within the long equitable temperature period. Storer (1925) and Zweifel (1955) have both presented evidence which would indicate that the onset of breeding in both *R. aurora* draytonii and *R. boylei* is correlated with water conditions in the environment much more closely than with environmental temperatures. Regarding *R. aurora* Storer (1925, p. 245) has said: "Temperature seems to have little to do with the initiation of spawning activities, as there is no conspicuous alteration in temperature coincident with egg deposition so far as data available to the author indicate. The only correlation which has occurred to the writer is that of maximum filling of ponds...". Storer is of the opinion that breeding in *R. boylei* is also strongly correlated with the water level in the streams in which they live. The relative stenothermy of these two species of frogs would also indicate adaptation to a mild, uniform climate. A comparison with the data obtained by Moore for the Eastern frogs graphically illustrates the narrowness of the temperature range of the two native California frogs.
<table>
<thead>
<tr>
<th></th>
<th>R. sylvatica</th>
<th>R. pipiens</th>
<th>R. salustris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower limiting embryonic</td>
<td>2.5°</td>
<td>5°</td>
<td>7°</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper limiting embryonic</td>
<td>24°</td>
<td>26°</td>
<td>30°</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryonic temperature</td>
<td>22.5°</td>
<td>23°</td>
<td>23°</td>
</tr>
<tr>
<td>range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. catesbeiana</td>
<td>R. aurora</td>
<td>R. boylei</td>
</tr>
<tr>
<td>Lower limiting embryonic</td>
<td>15°</td>
<td>9°</td>
<td>11°</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper limiting embryonic</td>
<td>32°</td>
<td>24°</td>
<td>26°</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Embryonic temperature</td>
<td>17°</td>
<td>15°</td>
<td>15°</td>
</tr>
<tr>
<td>range</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The embryonic temperature range of the two California frogs compares closest with that of R. catesbeiana, which has the most southern distribution of any of the Eastern ranids. This fact seems further substantiated by the apparent pre-adaptation of R. catesbeiana to conditions in California based upon its high degree of success since its introduction. The only adaptation to the new environment displayed by R. catesbeiana in California has been a wholesale shift upward of one degree of the embryonic temperature range.
Since the temperatures at which the *Rana aurora* embryos were raised were poorly controlled, the temperature coefficient for this species was not computed. For this reason computation of the temperature coefficient for the remaining species was deferred until complete data is available and comparison can be made.

Other than the shift in embryonic temperature range discussed above the data for the *Rana catesbeiana* from California apparently fits the data Dr. Moore has published for members of this species from the East. Until more information is available concerning the life history, distribution and ecology of this frog on the Pacific Coast, any discussion of the adaptive significance of the embryonic characteristics would be premature.
SUMMARY

The relationships between breeding habits, geographical distribution, and embryonic temperature tolerance in three frogs of the genus *Rana* occurring in California was investigated.

General conformity to the correlations established by Moore (1942a) exists between the two native California frogs: *Rana aurora draytonii* and *Rana boyleri boyleri*.

1) *R. aurora* has a lower minimal and maximal embryonic temperature tolerance than *R. boyleri*.

2) *R. aurora* develops more rapidly at lower temperatures than *R. boyleri*.

3) *R. aurora* begins breeding at an earlier time than *R. boyleri*.

4) The distribution of the *R. aurora* (subspecies) is more northern than the *R. boyleri*.

Certain aspects concerning embryonic temperature adaptations, distribution and breeding habits of the two native California frogs *R. aurora* and *R. boyleri* were discussed.

The comparatively slow rate of development and rather narrow range of embryonic temperature tolerance of *Rana aurora* and *Rana boyleri* was contrasted with the rates of development and embryonic temperature tolerances of some Eastern ranids. The significance of a slow developmental
rate and stenothermy of the embryos as adaptations to the unique climatic conditions of California was discussed.

The importance of availability of water as the most important limiting factor in the ecology of the California frogs was compared with the relationship of environmental temperature as the most important limiting factor in the ecology of the Eastern frogs.

The data derived from the temperature experiments with Rana catesbeiana embryos indicates a general shift upward of one degree in the embryonic temperature range, but little modification in the embryonic growth rate in comparison with similar data for Eastern R. catesbeiana.

In view of the paucity of information concerning the natural history of the introduced R. catesbeiana of the Pacific Coast discussion concerning adaptive significance of embryonic temperature tolerance was not considered feasible at this time.
LITERATURE CITED


---, 1949a. Geographic variation of adaptive characters in Rana pipiens Schrober. Evolution 3:1


Villeneuve, D.A. 1958. Temperature and the rate of development of the embryos of Rana pretiosa (unpublished Idaho Herpet Association undergraduate research paper, Department of Zoology, University of Idaho)
EXPLANATION OF PLATES

PLATE 1 and 2. (facing)
1. Photograph of equipment
2. Schematic diagram of equipment

PLATE 3.
Figure 1. The effects of temperature on the growth rates of embryos of Rana aurora.
Figure 2. The effects of various temperature on the growth rates of embryos of Rana boylei.

PLATE 4.
Figure 3. The effects of temperature on the growth rates of embryos of Rana catesbeiana.
Figure 4. The effects of temperature on the growth rates of embryos of Rana pretiosa. (from Villeneuve, 1958)

PLATE 5.
A comparison at various temperatures of the times to stage 20. Rana aurora, Rana boylei and Rana catesbeiana data from present work; data for Rana pretiosa from Villeneuve, 1958.

PLATE 6.
The growth rates of several Eastern frogs at various temperatures. (from J. A. Moore, 1939)

PLATE 7.
A comparison of times to stage 20 of several Eastern frogs. (from J. A. Moore, 1942b)
Plate 1. Photograph of equipment
Plate 2. Equipment diagram

Legend
1. Pump
2. Thermostatically controlled oil bath
3. Shell C. avus oil (anhydrous)
4. 160 mm crystallization dish filled with pond water
5. Water level
6. Aluminum divider strip
7. Coils of refrigeration unit
8. Compressor
9. Temperature gradient tank
10. Sending unit for thermostat controlling refrigeration unit
Plate 4

Fig. 3
R. catesbeiana

Time since stage 3

Stage of development

Fig. 4
R. pretiosa

Time from fertilization

Stage of development
Fig. 5

- $x$ = R. catesbeiana
- $\triangle$ = R. aurora
- $o$ = R. boylei
- $\square$ = R. pretiosa

Time to stage 20 vs. Temperature graph.
HOURS SINCE FIRST CLEAVAGE

RANA PIPIENS

RANA CLAMITANS

RANA PALUSTRIS

RANA SYLVATICA

(Reprinted from Dr. J. A. Moore, Ecology, Vol. 20, October 1939)
Plate 7

- CATESBEIANA
- CLAMITANS
- PALUSTRIS
- PIPiens
- SYLVATICA

Temperature vs. Hours to Stage 20