

XIX. HIBERNATION STUDIES I.  
BEHAVIOR OF RANA DURING THE HIBERNATION  
PERIOD.

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Hibernation is the term employed to denote the peculiar state of torpor in which many animals which inhabit cold or temperate climates pass the winter. It is generally understood as winter-sleep (German, "Winterschlaf": French, "sommeil hivernal"; and Italian, "letargo"). However, in hot or dry countries, various animals pass into a similar state during the hottest season of the year; this condition is called "aestivation." Several animals which hibernate during the winter may fall into a similar state at intervals during mild weather and Hall (1832) has applied the term "Diurnation" to the day sleep of bats which he regards analogous to hibernation. Parker and Thompson (1927) quote Wheeler as using the term "diapause" in speaking of this dormant period in some insects.

Hibernation is a means by which certain non-migratory species can live through unfavorable climatic conditions which would end fatally in starvation or desiccation were the animals to maintain their normal state of activity. It is a physiological condition, and not produced simply by cold alone, though it is favored by it, because cold induces sleep, which may afterwards pass into hibernation. During hibernation the temperature of the bodies of the animals sinks to a point corresponding nearly to that of the surroundings; but if they are exposed to an unusual amount of cold, they are first awakened by it, and then sink into a fatal torpor. Hibernation should not be confused with the torpor of freezing, which is accidental and usually fatal.

During the dormant period the vital processes are reduced. These changes are especially marked in certain mammals since they undergo sudden transformation marked in certain mammals since they undergo sudden transformation marked from homoiothermic to poikilothermic type. The environmental conditions associated with the resting state of animals are lack of food, low temperature, and drought. This process occurs in some forms in almost every large group of the animal kingdom. Protozoa and annelids commonly encyst; snails remain in their shells; fishes enclose themselves in a mass of mud, and poikilothermic land vertebrates go into water or burrow; while mammals which hibernate are insectivores, rodents and some carnivores.

The hibernating animal is able to endure cold, deprivation of food, confined air, effects of many drugs, and other conditions that would be fatal at other times. Respiration being almost suspended, the maintenance of vitality depends largely on the action of the heart, which will continue for a long time after the hibernating animal has been decapitated. Hall (1832) believed that the amount of respiration varies inversely as the degree of irritability of the muscle fiber. Every gradation may be met with between ordinary sleep, the imperfect or abnormal hibernation of some animals, and the profound hibernation of others in which all functions of life are suspended.

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Kuhne found *Amoeba* cooled to nearly 0° almost motionless. Purkinje and Vanetin (1857) first noticed that the ciliated epitheum of the frog ceased movements at 0°. Muscles of the frog were found by Kuhne to become rigid at 3°-7° but were not wholly lacking in irritability.

All Reptilia in cold or temperate climates hibernate. Land tortoises bury themselves in holes in the ground and fresh water tortoises in the banks of rivers and lakes. Lizards and snakes take themselves to holes in trees, under stones, in caves, or among dead leaves. They may congregate in numbers and pass the winter closely entwined in a more torpid condition than that of hibernating mammals, their digestion and respiration being suspended. When the viper is disturbed during the winter the bite is said to be harmless; but similar harmlessness is not characteristic of venomous serpents which aestivate in tropical countries.

Amphibia generally hibernate in masses in the mud at the bottom of the water, and if awakened from hibernation can remain considerably longer under water without drowning than frogs in breeding season. (Cleghorn 1910). The frog is a cold-blooded or poikilothermous type, the temperature being near that of the environment. When it is very cold the metabolism slows down and the frog becomes sluggish, often falling into what seems to be a deep sleep.

Most workers on the study of hibernation have stressed the cause of hibernation and the conditions necessary to arouse the animal from the torpid state. In this experiment there has been no attempt toward determining these facts other than conclusions drawn from observation of the behavior of animals due to changes in climatic conditions. The present paper is concerned with the activity during the time favorable for hibernation.

In order to study the frog constantly during a long period it was necessary to provide an artificial habitat. This habitat was made so that it would be as much like the natural environment as possible. A frame three feet by five feet by three feet was built of white pine. This was painted with white lead paint, and the sides, ends, and bottom were covered with screen wire. The lid was of pine. The cage was put into the ground, the lid being on a level with the soil. Earth was packed around the box except at one end where there was a hole left about one and one-half feet longer than the box so that there was plenty of space for water. The box was filled with a mixture of sand and earth, the surface of which sloped to within three inches of the top at the back end and ten inches from the bottom at the other end. An electric light was hung in the top of the box for use in attracting insects for food at night. This was used only during the first weeks. The earth within the box was saturated with water at all times, and until very cold weather a good supply of water was kept in the pond in the front of the box.

On November the fifth, 1926, fifty-eight *Rana pipiens* were received from Louisiana. They were marked, weighed, and put into the habitat, toes being removed as identification marks. The sphagnum moss in which the frogs were shipped was put into the box, and as it was full of insects there was plenty of food. These insects and those attracted by the electric light were the only food supplied except

some *Drosophila* cultures which were placed in the cage during the first three weeks of November. A recording thermograph was placed on top of the box.

In order to describe the climatic conditions under which the experiment was carried on a table is given including average velocity, and direction of the wind; maximum and minimum temperatures; humidity; pressure and daily precipitation. The readings were taken at two-thirty p. m., the station being on top of a three story building which is a little less than a block from the frog habitat.

The frogs were observed from time to time during the period between November the fifth, 1926, and March the twenty-third, 1927, and their behavior recorded. At intervals frogs were taken into the laboratory where they were weighed and dissected. A portion of each type of tissue was fixed for histological study later. The following procedure was decided upon and was carried out in the case of each frog. First the frogs were observed in the box without being disturbed. They were then punched with a stick and the reactions noted. A frog was selected which had not been disturbed, and taken to the laboratory. The excess moisture was removed with a piece of paper toweling. The frog was weighed on the same scales and in the same box in which it had been weighed November the eighth. It was then killed. The condition and appearance of the organs were observed and recorded. The organs and parts of other tissues were removed and put into a killing and fixing solution.

#### *Observations.*

During the course of the experiment the maximum and minimum temperatures were 25.6° C and 11.7° C, respectively. The total precipitation between September 25th, 1926, and March 23rd, 1927, was 97.3 centimeters. The temperature quoted in the following discussion are maxima.

From November 8th, 1926, until the last of November, the frogs were active. They could be seen through the screen wire, hopping in and out of the water. When the lid of the box was raised they would hop about wildly and go to the back of the box. The maximum daily temperature was, for the most of the time, around 18° C, although there was one cold period when the temperature was around 4.5° C for a few days. This seemed to make no change in the behavior of the frogs. Two frogs were killed, one whose weight was not recorded, and one which had gained 0.4 grams.

During the first three days of December the weather was warm but there followed several days when the temperature was around 4.5° C. More than two inches of rain fell and there was usually a wind from the north. About the eighth of December the frogs began to be less active. A frog weighed on this day had lost 3.7 grams. It was interesting to observe the frogs becoming more and more inactive. They were sluggish but would become active again after having been handled a few minutes. By December the thirteenth the frogs had become dormant. Most of them were under the moss and some were in the soft earth but had their heads and forelegs sticking out. Seemingly, they had scooped out small burrows large enough for their bodies. I placed one of the dormant frogs on his back in my hand. Its hind legs lay limp while the fore ones were bent forward and were

not relaxed. The only movements the frog made were for balancing as I turned my hand from side to side. A frog weighed on December 30th had gained 0.67 grams.

The temperature rose from 15.5°C to 22.2°C between January 2nd and 6th. The weather was beautiful, often reminding one of late spring days. The frogs all came out from their shelter of moss and seemed to be as active as when they were put into the habitat. Then, there came a sudden drop to 4.4°C by the 13th but the frogs did not all find cover. About half of them huddled in the corner of the box and on the morning of January thirteenth, thirteen were dead.

On January 13th, 1927, a frog was taken from beneath the moss. It was dormant and cold but awoke before I had time to walk upstairs. This frog had lost 2.58 grams. One week later, when the temperature was around 7.2°C the frogs seemed to be in a heavy sleep. One taken on the twentieth did not awaken until after it had been carried upstairs and weighed, which was a matter of several minutes. It had lost 4.28 grams. After the box in which it was weighed was opened the frog climbed slowly out of the box and managed to stagger around on the table, never attempting to hop. The blood appeared to be dark in color and there seemed not to be as much as usual. The eggs were very small.

After the 22nd of January, the weather became warmer again, the temperature being around 16.6°C until after the 30th, but dropped four degrees by the 31st, after which it began to rise again. The frogs were dormant but awoke in less time than during the previous two weeks. They seemed to stretch when disturbed, and one, as it was near the side of the box, stood on tiptoe on its hind feet with fore feet clinging to the screen wire. It, and others, appeared to be actually stretching. A frog which had lost 3.95 grams was weighed on January 31st.

During the first week of February, the weather was warmer than usual, the temperature being around 16.1°C, but reaching 24.5°C on the 3rd. The frogs were awake but inactive. They did not move voluntarily but would hop when punched with a stick. On the 4th they had become slightly active. Two frogs were weighed, one on the second and one on the fourth. The former was the least active. It had lost 5.21 grams. The latter was lively enough to scratch around vigorously in the box while being weighed but it had lost 7 grams. The skin of the frog was tough; the abdominal muscle had adhered to it and there was difficulty in detaching it without the aid of scissors. The blood was bright red in color and flowed freely, giving the appearance of being thin.

By the sixth, the frogs had become so active that there was difficulty in catching one. Three frogs were killed on this day, two females and one male. One of the females had lost 3.9 grams and had no fat-bodies, while the other had gained 1.905 grams and had fat-bodies which were very pale in color. The liver of this frog was also light in color. The male had lost 4.945 grams. The fat-bodies were very large and the testes appeared to be longer than normal and decidedly pointed at the posterior end.

Between the seventh and ninth of February, the temperature fell from 19.5°C to 2.2°C. During these days the frogs went under cover

again and became quiet. They did not appear dormant as they had when they were previously inactive, but did not move about at all. Although the temperature began to rise again after the tenth the frogs did not become active. On the twelfth of February when the temperature was between 7.2°C. and 10°C, the frogs were very quiet and did not attempt to escape when disturbed. A specimen taken on this day had lost 3.3 grams. Only one small fat-body was found. The gall bladder was larger and very much darker in color than had been those of frogs taken previously. The eggs were large and abundant.

The week following the twelfth, the temperature rose to 25.6°C. on the sixteenth but dropped suddenly to 0.6°C. on the eighteenth. The frogs were quiet, not at any time making an attempt to hop. It was of peculiar interest to observe their attempts to walk. The process was slow and feeble and seemed to require a great deal of effort. The fat-bodies of the frog killed on February the nineteenth were very small; the eggs were abundant. This frog had lost 4.55 grams.

There was very little change in the behavior of the frogs for the next ten days. They were quiet for the most part. Some of them would hop about when disturbed while others would allow themselves to be pushed and tossed around, their movements being merely feeble efforts to right themselves when left on their backs. A specimen weighed and dissected on February the twenty-third had lost 7.3 grams. The fat-bodies were large and of deep orange color. They appeared to be bloody within while other organs seemed to be bloodless. There was some food in the stomach.

Two frogs, one male and one female, were dissected on March 6th, 1927. They were both quietly sitting under the moss about six inches from each other. The female had lost 6.42 grams. There were no fat-bodies found but the blood was plentiful. The spleen was unusually large and of a very dark reddish brown. The large intestine was stuffed with food material. The kidneys seemed slightly pale in color. The male had gained 4.5 grams. The entire length of the large intestine seemed to be crowded with food material. The fat-bodies were soft and filiform. The blood was thin.

It was not until March 15th, 1927, that another frog was killed but they were observed from time to time. They grew slowly more and more active. Although the ground was saturated, the pond was half filled with water. The frogs were seen sometimes in the water and sometimes out on the ground, but when the lid of the cage was raised they would all jump into the water. On the 15th one of the remaining frogs was weighed and found to have lost 3.25 grams. This one was very active. The organs, to all appearances, were normal except the fat-bodies which were not present.

The last of the experimental frogs killed was taken March 20th, 1927. The weather was somewhat colder so the frogs were under the moss about three inches from the pond of water. They were all huddled together but there was no sign of amplexation. When they were uncovered they were quite active and some jumped into the water. If they were not disturbed further they would scratch down into the moss again. The frog killed had gained 4.98 grams. The fat-bodies were filiform and the eggs were few and small. The entire digestive tract was full of material. This frog was very ac-

tive, being able to escape several times before I was successful in decapitating it. This was the first case of a frog giving any liquid excretion from the kidneys since December.

Unfortunately an accident occurred which brought the experiment to a close at this point. On April 10 a specimen which was undoubtedly fully active was obtained from the river. This frog was caught at "the falls," as it is locally known, which is about three and one-half miles southeast of Norman, on a tiny tributary of the South Canadian River. It was very active swimming in the pond below the falls. The frog weighed 43.48 grams. The organs appeared to be normal. The eggs were large and abundant. The kidneys were of a delicate orange color, and the entrance of the blood vessels was very pronounced.

### *Summary*

1. A study of the changes in the weights during hibernation gave conflicting results. Out of eighteen frogs, thirteen had lost weight. The most lost by any one frog was 7.3 grams. This was a female killed on February 23rd. Thirteen frogs had lost a total of 60.455 grams making an average of 4.65 grams loss each. One of these was a male. This male was killed February 6th; it had lost 4.945 grams.

The remaining five frogs, two males and three females, had gained. Males II and XVII, killed on November 23rd and March 6th had gained 0.4 grams and 4.5 grams, respectively. The females which had gained were killed on December 30th, 1926, February 6th, and March 20th, 1927. These frogs had gained 0.67 grams, 1.905 grams and 4.98 grams respectively.

2. The degree of dormancy was the most profound when the temperature was lowest. A rise in temperature seems to arouse the dormant animal even though it does not feed.

3. Time of first feeding was around February 23rd, as a little food material was found in the stomach. However, the entire digestive tract was not crowded with food until March 6th.

4. A fall in external temperature generally causes a frog to become quiet, burrow under cover and become dormant. Should the external temperature suddenly rise to its previous height, the animal awakens. A sudden rise in the external temperature leads to a slow awakening and then a sudden regaining of activity.

5. In one case an astonishing amount of moisture was produced while the frog was being weighed. The frog was dormant but became active during the time it was in the tin box on the scales. The frog and the entire inside of the box was covered with drops of moisture. No explanation was attempted as the condition did not occur again.

6. No liquid excretion from the kidneys was observed from the time the frogs were dormant until they became active in the spring.

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