Experimental Studies on the Developing Eggs.

I. Age and Environment in Amphibia.

By

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with 22 Tables, 2 Figures and 2 Plates.

I. Introduction.

Setting aside accidental affairs, the environmental factors, which influence the development of an animal, may be taken as consisting chiefly in food-supply, temperature, moisture and illumination. So far as concerns a water-inhabitant, the affection has little to do with the last named factor and not at all with the last but one; in a developing egg the food-supply from exterior is, of course, out of question. *Now the temperature must be regarded as the only factor which may affect the development at any rate.*

Besides the factors just enumerated, the oxygen-content in the surrounding water is to be taken into account, inasmuch as it turns, of course, striking influence to the speed, in which the eggs evolve. But it is ruled directly by the temperature of the water and may consequently be calculated from the latter, so that the latter can be designated as the sole agency that has influence upon the development. This is the reason why the present observation has been carried on with the eggs layed in the water, and I have employed the Amphibian eggs not only because they are abundant in the waters about Sapporo, but because the facts concerning the development of them are made best known, so as to be convenient enough in comparison to those obtained from the experiments of the present work.

The eggs employed as the material are represented by only two species; nevertheless they are the representatives of the two Amphibian Orders: Urodela and Anura. In particular, one of them, *Rana fusca*, one which is best studied, and the other, *Hynobius lichenatus*, is a

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member of Urodela, which is not only looked upon as one of the most primitive form, but, as quite recently made known by Dr. SASAKI, is the only example except Axolotl, which brings offsprings in the larval stage.

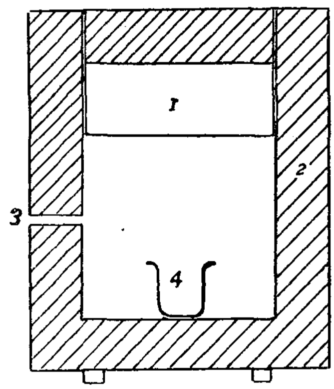
With great interest I attempted the present experiment to see how far the results of it elucidate the Amphibian development in this contrast of the two forms in the zoological scale.

Observed in Sapporo, the spawning of the two species shows them to agree practically in season. Last spring, at which time the material was collected, it began in *Rana* on the 10th of April and in *Hynobius* the 7th and closed in the former the 22nd, and in the latter the 21st, of the month, so that it lasted some two weeks in both the forms.

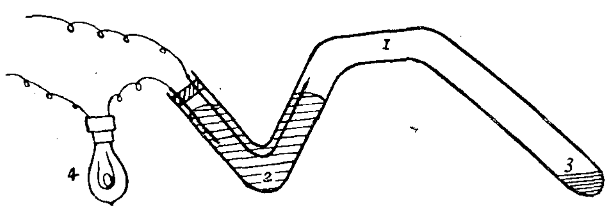
The lowest temperature, in which the eggs were to develop, was at 0°C and they were brought over into the medium of 5°C, to be, then, put in that at 10°C, and to be exposed finally in the highest temperature of the present experiment, which was at 15°C. As the atmospheric temperature changed from time to time during the course of the two weeks in which the experiments had been carried out, it is indispensable to equip a system of apparatus for keeping the temperature which stands under the control of the observer. For this purpose I prepared a series of refrigerators. According to the four grades of temperature mentioned above, in which the eggs were to be exposed, I have used four such requisites of experiment, one of which was for 0°C and was substituted by an ordinary refrigerator, whereas the remaining three were of wooden boxes prepared after my own design (*Text-fig. I*). All three boxes have an internal chamber which measures 1 foot for all three dimensions; the walls of which are formed of double wooden plates, contain saw-dust in the spaces and are 7 inches thick in one box for the temperature of 5°C and some 4 inches in the other two, while the internal surface of the walls is tapestried with woolen cloth of green color. In this construction the internal chamber was proved to be absolutely shut off from the thermal influence of the exterior.

As the thermal source in the internal chamber I employed an electric lamp which stands in connection with the thermoregulator of my own design. The latter consists of an S-shaped glass tube (*Text-fig. II*); the distal section of the tube (3) which is connected with its blind end, contains ether, the middle section (1) is simply vacuous, and the proximal section (2), which is filled with mercury, is stopped at its end with seal-rubber, through which the two wires enter into. One of the wires ends naked shortly at its entrance, whereas the other emerges on the distal surface of mercury and terminates also naked. It is this termination of the wire which closes the electric circuit when the vapour-pressure of the ether is diminished so as to swell up the mercury which comes to cover the naked end of the wire, controlling the temperature of electric heat in the chamber, which can easily be examined by means of the thermometers of the apparatus capable of being drawn out at necessity.

Text-fig. I.



Text-fig. II.



Text-fig. I. Cross-section of refrigerator.

1. metallic box containing ice-pieces.
2. double walls of wooden plate, filled up with saw-dust in spaces of them.
3. hole for thermometer and electric conducting wires.
4. glass-vessel for eggs to be experimented.

Text-fig. II. Diagrammatic longitudinal section of thermoregulator.

1. middle section of glass tube free from atmospheric air and full of etheric gas.
2. proximal section of glass tube with mercury.
3. distal blind end of glass tube containing ether.
4. electric lamp as heat source, standing in connection with glass tube.

The eggs contained in glass-vessels are put in the chamber, where the temperature can be regulated approximately at the same grades in the air and water. In the field, the atmospheric temperature varies according to days observed, as shown in the following table:

Table I.

The atmospheric temperature in April.

date	temp. C°	date	temp. C°
1st	6.39	16th	4.33
2nd	7.31	17th	5.81
3rd	4.65	18th	4.40
4th	3.78	19th	3.88
5th	3.41	20th	7.66
6th	0.45	21th	9.29
7th	1.05	22nd	10.43
8th	1.73	23rd	11.63
9th	3.84	24th	7.43
10th	6.53	25th	9.75
11th	6.85	26th	12.06
12th	6.72	27th	12.38
13th	8.24	28th	9.93
14th	7.88	29th	10.53
15th	6.90	30th	10.43

Nevertheless, the water temperature was not much influenced from the changes in the atmosphere; it ranges from 5°C to 7°C.

In this opportunity I may be permitted to express my warmest thanks to Professor HATTA who has not only caused me to undertake the experiment, but been so kind constantly to help me in forwarding the work. My obligations are also due to Dr. SASAKI for his kindness in putting his unfinished material of *Hynobius lichenatus* at my disposal. I am also obliged to thank to Dr. OGUMA and Mr. SHINJIO for their courtesy shown to me during the course of the present work.

II. Experiments.

The present experiments have been carried out in the following way. In all the cases the experiments have been commenced with the eggs which were 5 hours old and closed at the end of the 8th day of the experiment. The series of the combinations of the time and temperature, in which the eggs have been exposed for development are given in the following pages.

A. Hynobius lichenatus, Boul.

For the convenience of reference we shall first of all nominate the stages of development (*Table II*).

Table II.

Stage 1. 4 cell stage.
Stage 2. 8 segment stage.
Stage 3. 16 segment stage.
Stage 4. 64 cell stage.
Stage 5. Young morula stage, in which the macromeres are enormous in size in sharp contrast to the small micromeres occupying the small area around the animal pole.
Stage 6. An advanced morula stage; the macromeres are divided into small segments so as to diminish the difference between macro- and micromeres and the micromeres spread themselves over the animal hemisphere.
Stage 7. The stage which is usually regarded as the blastula.
Stage 8. The stage of commencing gastrulation.
Stage 9. An advanced gastrula stage, in whieh the animal layer is reflected inwards, so as to show a sharp demarkation against the vegetative field (*Fig. 1*).
Stage 10. The old gastrula stage with the invaginated furrow encircling the yolk-plug.
Stage 11. The oldest gastrula stage, in which the yolk-plug and blastopore are reduced into a mere spot, and the dorsal furrow (Rückenrinne) comes into view (*Fig. 2*).
Stage 12. The stage in which the blastopore is closed to its last rudiment, and the medullary folds are about to appear in

the anterior part (*Fig. 3, a, b*).

Stage 13. The stage with the distinct medullary folds, and the dorsal furrow (Rückenrinne) fades to a great extent (*Fig. 4, a, b*).

Stage 14. The stage with the medullary folds brought close to each other (*Fig. 5, a, b, c*).

Stage 15. The stage in which the fusion of the medullary folds on both sides are taking place, and 4 pairs of the mesodermic somites are externally visible (*Fig. 6, a, b, c*).

Stage 16. A young larva with the head-protuberance crooked downwards and furnished with 3 pairs of gill-slits; 6 pairs of the mesodermic somites are externally obvious (*Fig. 7, a, b*).

Stage 17. The stage in which the eyes, 4 pairs of gill-slits and 12 pairs of the mesodermic somites are visible externally; the tail with the caudal fin is striking (*Fig. 8*).

Stage 18. The oldest larva seen in the present work; besides the eyes and gill-slits, the stomodaeal depression and mandibular arch are very obvious; the head, trunk and tail are prolonged considerably (*Fig. 9*).

The eggs contained in a jelly-sack[1], which were from 25 to 37 in number, have been regarded in the experiment as a set.

The First Case.

The first set was put in 0°C, the second set started at 5°C, and in the third set the work was commenced at 10°C, whereas the fourth was in the box of 15°C. In all the sets the eggs were examined at the close of every 24 hours from the first day until the eighth, in which they stayed, and the results are given in the following table[2].

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1) It is very well known that in the salamander a spawn consists of a pair of long jelly-lobes or jelly-sacks, one end of which is stuck to a foreign body such as fallen tree-twigs, water-plants etc. submerged in water.

2) The table gives the results of the experiments carried out from 11th to 30th of April last. The figures show the standard stages above defined. The case younger or older than the stage corresponding to figures given are indicated respectively by (−) or (+).

Table III.

	I.	II.	III.	IV.
Time examined \ Temp.	15°C	10°C	5°C	0°C
1st day	6	5	3	1(−)
2nd day	8(−)	7	4	2(−)
3rd day	9(+)	8	6	2
4th day	12(−)	9	7	4
5th day	16(−)	10	8(−)	5(−)
6th day	16	11	8	5
7th day	17	13	9(−)	5(+)
8th day	18	15	9(+)	6

As seen in the table given above, the influence of the temperature upon the eggs is striking. *In particular, it is noteworthy that the age expressed by grades of development advances by stages.* The stage at the end of the first day (column I) which started at 15°C is approximately attained by the eggs of 10°C at the close of the second day (column II) and by those of 5°C only at the end of the third day (column III), while in the eggs of 0°C this stage corresponds to that of the final day of experiment (column IV). The stage of the second day of the first column is found in the eggs of the second and third columns respectively in the third and fourth day, and it is no longer represented in the fourth column. The stage of the thrid day of the first column is met with in the second and third columns likewise in the fourth and eighth day. The stage of the fourth day of the first column is represented, on the other hand, neither in the fifth nor in the sixth day of the second column, but falls between the sixth and seventh day of it, and it is found no longer in the third column at all. In the same way the five days old stage of the first column is found at the close of the eighth day of the second column; and there are represented even in the second column no stages that agree with the stage of the sixth, seventh and eighth day in the first column.

The Second Case.

a. Ascending Series.

In this series of experiment a set of the eggs has been carried over from a certain temperature, at a certain interval of time, to higher grades of it, until it was put in the highest temperature, 15°C. According to the temperature at which the development started, and to the duration in which the process went on, the series are divided into five groups. In the *fisrt group* the eggs started to develop at 0°C and stayed two days in each of the scales of temperature given above, e.g. in 0°C, 5°C, 10°C and 15°C, so that the days spent made a total of eight days. The *second group* of the eggs started likewise at 0°C and jumped into 15°C, staying 4 days in each medium. In the *third group* they developed 64 hours (8/3 days) respectively at 5°C, 10°C and 15°C. The *fourth set* spent 4 days in 5°C and jumped into 15°C where it stayed the other 4 days. Lastly, the *fifth set* was exposed every 4 days in 10°C and 15°C. The results of the experiments are shown in the *Table IV.*

Table IV.

	I.	II.	III.	IV.
Temp. / Time elapsed	0°C	5°C	10°C	15°C
Every 2 days	2(−)	6	7(+)	13
Every 4 days	4	—	—	13(+)
Every 8/3 days		6	8	16
Every 4 days		7	—	16
Every 4 days			9(−)	16(+)

In this series two sections can be distinguished in regard to the stages; in the younger section, the oldest stage at the end of the eighth day corresponds to the stage 13 of age (see *Table II*), and in the older section the oldest stage is at the stage 16 of age. Whilst the former was attained at the end of 7th day, when the eggs have developed throughout even in 10°C (see *Table III*), the latter corresponds to the six days old eggs which have developed always in the medium of 15°C. In spite of the temperature, in which the development went on, the process so retarded, as it is quite obvious, only because it suffered from the influence of the long exposure in the lower temperature.

The stage of 10°C in *Table IV*, e.g. the stage 7 and 8, at which the eggs arrived in the 6th day, are shown in *Table III* to have been attained in 2 and 3 days; the stage of 5°C in *Table IV*, e.g. the 6th or 4 days of age, is represented in *Table III*, to be 3 days old. It is evident that this delay of development is due to nothing else than the lower temperature in which the eggs were exposed beforehand. Lastly, it is self-evidently due to the same agency that the eggs of the first column of *Table IV* have developed from the stage 4 to the stage 13, and those of the second column from the stage 7 to the stage 16 by jumping progress.

From the facts above given, preliminarily it is seen now that the temperature throws its strong influence upon the progress of development.

Next comes in consideration the case in which the course of the development of 8 days has been equally divided into 2 or 3 intervals of time to be exposed in the temperature varying from 0°C to 10°C or 5°C (*Table V*).

Table V.

Temp. / Time elapsed	0°C	5°C	10°C
Every 8/3 days	3	6	9(−)
Every 4 days	4	—	9(−)
Every 4 days		7	11(−)

The *first set* developed the first third of 8 days in 0°C, the second third in 5°C and the last third in 10°C. The stage attained in 8 days has been equal to that of the eggs developed 4 days from the first in 10°C (*Table III* and *IV*). The difference of 4 days in this case is of course owing to the lower grades of development which was attained by eggs at the moment carried over into the medium standing in question, e.g. 10°C. The stage in question is in turn younger than that proper to it, e.g. the stage 6 of age which must properly be attained at the end of the third day (see the third column of *Table III*); the latter stage is delayed because the preceding stage, the third stage, was younger than the stage due to it, the fourth stage, in consequence of the lower temperature in which the eggs have been exposed to develop.

The *second set*, in which the eggs jumped from 0°C to 10°C, has undergone the same influence of the temperature and attained the same stage as the first set, because they stayed, in this case, longer in the higher temperature than the latter. In the *third set* the influence of the temperature is still more obvious: for the last stage of it represents only the stage of the 6th day.

In the next case (*Table VI*), the eggs were admitted to develop 4 days in 0°C and the other 4 days in 5°C; and the stage of 6th day is to be seen only at the end of 8th day.

Table VI.

Temp. / Time elapsed	0°C	5°C
Every 4 days	4	8(−)

The results arrived at in the experiments of the prolonged intervals of time and at the maximum temperature lowered are quite striking to show the strong influence of temperature.

b. Descending Series.

The experiments of the present series have been carried out in parallel with those of the preceding series, that is to say, of the ascending; the difference between the two series consists in the direction, to which the eggs were transferred, and which is carried over inversely from higher temperature to lower one.

As in the ascending series, the experiments were grouped into five classes. In the first class, a set of eggs commenced their development at 15°C and was carried over successively in 10°C, 5°C and 0°C, being exposed 2 days in each temperature. In the second class, the development lasted 4 days in 15°C and 4 days in 10°C. The set of the third class was put 8/3 days in 10°C and brought over successively in 5°C and 0°C, in each of which two media it was exposed likewise 8/3 days, while the objects of the fourth class spent 4 days in 10°C and jumped into 0°C, where they spent the remaining 4 days. In the last class the commencing development of 4 days went on in 5°C and the latter half of it in 0°C. The results are given in *Table VII.*

Table VII.

	I.	II.	III.	IV.
Temp. / Time elapsed	15°C	10°C	5°C	0°C
Every 2 days	8(−)	9(+)	11(−)	11(−)
Every 4 days	9(−)	—	—	9(−)
Every 8/3 days		8	9(−)	10(−)
Every 4 days		9	—	9(+)
Every 4 days			7	8(−)

In these groups of experiment there are two prominent facts which are worth of note: in the first place, *the progress of development* is, though slow, yet *remarkable,* when the temperature was changed gradually, as seen in the first, third and fifth sets of eggs (in the first, third and fifth transverse rows of *Table VII*). In the second place, the development was *entirely* or *almost given up,* when the change of temperature took place suddenly, as is quite obvious in the second and fourth sets of eggs (in the second and fourth transverse rows of *Table VII*). While in the former case the *time that elapsed is to be taken as an agency for the development, it has, in the latter, no effect upon the stage* which is expressed by the grades of development. It is evident enough that the sudden change of temperature decreased the vital activity into the minimum or 0, so that the eggs were to be dormant. On the contrary, in the case of gradual change the development was carried on, because the vital activity was, though diminished, kept up. This fact is shown further by the group of experiments which follows.

A comparison of the two cases of descending series shows that the sudden change of temperature is, as seen in the preceding case, not whole of the cause which stopped the progress of development. *The dormant state must in this case have been brought about not by the sudden thermal change itself, but because the eggs were suddenly carried over in the medium of 0°C.* It is, therefore, evident that the last named medium threw *its maximum negative efficacy upon the eggs which were acted upon suddenly by this agency;* its affection by grades causes the negative efficacy only to be increased.

The groups which come next in question are divided into 3 classes, in the first and second of which the eggs started to develop at 15°C, while in the third class they start at 10°C. In the first class the eggs spent every 8/3 days successively in 15°C, 10°C and 5°C, and in the third class they stayed for the former 4 days in 10°C and for the latter 4 days in 5°C, while the set of the second class jumped from 15°C to 5°C, spending 4 days in each. *Table VIII* shows the results of the work.

Table VIII.

Temp. / Time elapsed	15°C	10°C	5°C
Every 8/3 days	9	13	16(−)
Every 4 days	9 ?	—	10(+)
Every 4 days		9	12

For the first and third sets which were both brought over by a difference of 5°C into lower temperature, it is only to be noticed that the stage at the end of the eighth day shows a striking progress, as compared with that in the corresponding time elapsed; this is because of higher temperature where the development went on. But in the second class where the temperature was changed suddenly by 10°C, the *progress of development did not totally stop, but stands inferior to the first set* (compare the second transverse row with the first in *Table VIII*).

c. *Reverting Series.*

In these series two cases have been experienced: in the first case the objects stayed in a certain temperature and were transferred into a lower, to be again brought back to the original temperature, at which the development commenced its work; in the second case the course was taken in the inversed direction, that is to say, the objects come from a lower temperature to a higher and were again taken back to the lower temperature. As the course of 8 days was divided into three equal sections, the duration of time in which the eggs developed at a certain temperature was equally 8/3 days. In the *first case*, the five classes are distinguished. In the first, second and third classes the higher temperature was always represented by 15°C, while the lower temperature were set respectively at 10°C, 5°C and 0°C. In the fourth class the higher temperature was at 10°C and the fifth class at 5°C; the lower temperature was in the former class at 5°C and in the latter 0°C. The results of the experiments are in the annexed table.

Table IX.

	I.	II.	III.
Every 8/3 days	15°C	10°C	15°C
	9(−)	13	18
	15°C	5°C	15°C
	9(−)	9(+)	16(−)
	15°C	0°C	15°C
	9(−)	9	14(−)
	10°C	5°C	10°C
	8(−)	8(+)	12
	5°C	0°C	5°C
	6	6	8(+)

From the table it is seen that in all these classes the progress of development is almost in parallel with the grades of temperature and the duration of time, in which the eggs were exposed.

In the *second case*, there are two classes, in one of which the temperature at the commencing development was at 0°C, and the intermediate temperature at 15°C, whilst in the other class either temperature was set respectively at 5°C and 15°C. The results of the experiments are seen in *Table X.*

Table X.

	I.	II.	III.
Every 8/3 days	0°C	15°C	0°C
	3	5	5(+)
	5°C	15°C	5°C
	6	11(−)	12

In this case the influence of temperature and the duration of time in which the development went on is so obvious that a further argument thereon is hardly necessary.

On the other hand, there is seen in both cases (*Table IX* and *X*) a remarkable fact as regards the development in relation to the temperature in which the process went on, as the following illustration shows.

In the first class of the first case (*Table IX*), the oldest stage is exactly in correspondence with that of the eggs which developed throughout all 8 days in the higher temperature, e.g. 15°C (compare with column I of *Table III*); it follows that the influence of the lower intermediate temperature is not here expressed at all; it had no efficacy on the development. In the second and third classes (class of 15°–5°–15° and that of 15°–0°–15°), the lower temperature had only a weak influence, inasmuch as the oldest stage in either case is exactly (in the former case) or approximately (in the latter case) equal respectively to the stage of the 6th and of the 5th day of the eggs exposed throughout in the higher temperature, 15°C. In the two other classes (class of 10°–5°–10° and that of 5°–0°–5°), a feeble influence of the intermediate temperature is also recognized; the final stage attained, in the former case, about the stage of 7th day, and in the latter case, exactly that of 6th day of the eggs put throughout the course of time in higher temperature, e.g. 10°C or 5°C.

In short, it is seen that the *predominant temperature regulates the development, whereas the intervening temperature has very little or no effect upon it.*

The same argument is valid in the second case (*Table X*). In the *first* of the two classes, the intermediate higher temperature (15°C) came forth very a little, as seen from the oldest stage of the class, which could not attain the stage at the end of the first day of development in the same thermal medium (compare with column I, *Table III*). In the *second class* this stage is represented only by the eggs which were 4 days in the intermediate temperature.

From the reverting series of experiment we see, *therefore, that a short course of time has little influence upon the development;* in the other words, *the duration of time is to be regarded as a strong factor.*

B. Rana fusca, Thom.

The next experiments have been made with *Rana fusca* in parallel with those of *Hynobius lichenatus.* So far as concerns the early development, the results from the observation confirm those from the preceding species. It is, however, not uninteresting that there is a striking difference between the two species, which is in connection with the progress of development. A stage attained by the Urodel at a certain temperature has been revealed in the Anura in about half an interval of time; hence the development in the present case must be taken to go on very much swifter than in the foregoing case. Accordingly, the ages ascertained above can not directly be applied to Rana as the standards of comparison in the advancement of development; the following arrangement is tabled in this respect for this species.

Table XI.

Stage	1.	2 cell stage.
Stage	2.	4 cell stage.
Stage	3.	8 cell stage.
Stage	4.	16 cell stage.
Stage	5.	32 cell stage.
Stage	6.	The young morula stage in which the micromeres

are divided into numerous cells, in a sharp contrast to the large sized macromeres.

Stage 7. The blastula stage of the segmentation.

Stage 8. The stage at the beginning of gastrulation.

Stage 9. An advanced gastrula stage, with the crescent-shaped furrow of blastopore (*Fig. 10*).

Stage 10. The old gastrula stage, the invaginated furrow of blastopore completely encircling the yolk-plug (*Fig. 11*).

Stage 11. The oldest gastrula stage in which the yolk-plug and blastopore are reduced to a spot, and the dorsal furrow (Rückenrinne) commences to appear (*Fig. 12*).

Stage 12. The stage in which the medullary folds begin to appear, the dorsal furrow being to fade (*Fig. 13*).

Stage 13. The stage with the strongly raised medullary folds, and gill and sense plates are about to appear (*Fig. 14*).

Stage 14. The stage in which the medullary folds are closed to each other (*Fig. 15*).

Stage 15. The stage with completely fused medullary folds and distinctly differenciated gill and sense plates (*Fig. 16 a, b*).

Stage 16. The young larva furnished with a pair of suckers, 3 pairs of gill-slits and the elongated tail-bud (*Fig. 17*).

Stage 17. The stage in which the eyes and the gill-plates are expressed distinctly, the externally visible mesodermic somites are about 13 in number and the continuous dorsal and caudal fin fold is striking (*Fig. 18 a, b, c*).

Stage 18. The larva with a stomodaeal depression and 2 pairs of undifferenciated external gills; tail-bud with caudal fin prolonged in considerable length (*Fig. 19*).

Stage 19. The oldest larva in the present experiment, which is about to hatch; besides well developed suckers, the stomodaeal depression and the nasal pits, there are seen the further developed gills and the prolonged tail (*Fig. 20*).

The older lot of the oldest larva in the present work; the development of the gills, tail etc. are developed further than in a larva from the younger lot (*Fig. 21*).

THE FIRST CASE.

As in the foregoing series of the experiments, the eggs have been divided into 4 sets, each of which have developed throughout 8 days at a constant temperature that is represented respectively by 15°C, 10°C, 5°C and 0°C, and the results from these experiments are given in the following table.

Table XII.

	I.	II.	III.	IV.
Time examined \ Temp.	15°C	10°C	5°C	0°C
1st day	7	6	5	2(−)
2nd day	11	8	6	3
3rd day	14	9(+)	6(+)	5
4th day	16	11	7	6(−)
5th day	17	11(+)	8	6
6th day	17(+)	12	8(+)	6(+)
7th day	18	14	9	6(+)
8th day	19	15	10	6(+)

Before proceeding further, it is to be mentioned that *the set exposed in the medium of 0° C died, before the course of 8 days had been accomplished.* It is evident that this case was not accidental, as seen from the fact that all the eggs put in the same medium of temperature died without exception of a single egg, and that there was no trace of suffering from zygotic cause. There is, therefore, little room for doubt that the extreme cold allowed the egg to live so long, the death having probably taken place at about the end or at least in the course of the sixth day, as it is clearly seen that the progressing development is obvious up to that date. It follows that *the eggs are not only checked to develop, but are killed by their still longer exposition in severe cold.*

So far as it seems to me, the fatal action of the lower temperature is due to the development which went on very energetically in the present case. A comparison of the stages of a column of higher temperature with those of a column of lower temperature in *Table XII* shows, how sensitive to the thermal agency the Anuran eggs are; difference in stages between the given two columns is much more striking as compared with that in the Urodel. Otherwise I have little to add to the corresponding case of *Hynobius*.

The liability of the Anuran eggs to the influence of the temperature is still more clearly illustrated by examination of the respective grades of development in the course of the first and latter 4 days. The Anuran eggs, for instance, of the fourth day (column I—IV, *Table XI*) are advanced in development beyond the Urodelan of the 5th day (column I—IV, *Table III*); divergence in this respect between the two forms comes forth still more strikingly in younger stages, for instance, in the 3rd and 4th days. A further detailed analysis of a stage attained in 24 hours shows, however, how efficacious higher as well as lower thermal agencies are. As shown in the annexed table (*Table XIII*), at lower temperature, for instance, at 0°C or at 5°C (column III, IV), the progress of the development is practically not recognizable in spite of the long extent of time, while at the higher temperature of 10°C and 15°C (column I, II) it is remarkable.

Table XIII.

	I.	II.	III.	IV.
Time examined \ Temp.	15°C	10°C	5°C	0°C
2nd hour	1(−)	1(−)	1(−)	1(−)
3rd hour	2(−)	2(−)	1(−)	1(−)
4th hour	3	2	1(−)	1(−)
6th hour	5	3	1	1(−)
24th hour	7	6	5	2(−)

In the course of the latter 4 days, on the contrary, the development retarded so strikingly that under lower temperature the difference between the two Amphibian forms is scarcely or not at all recognized, as evident at the close of the 8th day (compare *Table XII* with *Table III*). The retarding development is more striking towards the lower grades of temperature and goes at last no further, so that in the medium of 0°C the cold caused, as above said, even a mortal effect on the developing eggs. Then it is evident that the medium of 0°C does not allow the Anuran eggs in the field to develop any further than the stage 6 of age.

The results from the present case may be, as it seems to me, *due to the physiological activity of the eggs, which grow less towards lower temperatures and under the most unfavorable thermal medium with a long extent of time*. In short, the efficacy of thermal influence in Anura exceeds to greater extent than in Urodela.

The Second Case.

a. Ascending Series.

The experiments of this series have been carried out in the same way as in *Hynobius*. In the first group, the eggs which started to develop at 0°C, stayed in each scale of temperature, e.g. 0°C, 5°C, 10°C and 15°C, and the results attained at the end of the 8th day are brought out in the first transverse line of the following table (*Table XIV*). The eggs of the second group jumped from 0°C in which they have developed 4 days, into 15°C to spend therein the remaining 4 days, and the results are given in the second transverse row of the table (*Table XIV*). In the third group the eggs were left to develop 64 hours (8/3 days) in each medium of 5°C, 10°C and 15°C respectively, and the results are seen in the third transverse row of *Table XIV*. The fourth group of eggs were put the first 4 days in 5°C and carried over into 15°C in which they spent the other 4 days; in the fifth group the eggs have developed 4 days in 10°C and 15°C respectively; and the results are seen in the fourth and fifth rows of the table above referred to (*Table XIV*).

Table XIV.

	I.	II.	III.	IV.
Time elapsed \ Temp.	0°C	5°C	10°C	15°C
Every 2 days	3	6	9	15
Every 4 days	6(−)	—	—	18
Every 8/3 days		6(+)	10	17
Every 4 days		7	—	16
Every 4 days			11	19

Here the influence of temperature is still further prominent, as a comparison of *Table XIV* with the corresponding case in *Hynobius* (*Table IV*) shows. Either the stages in earlier phases of development or those at the end of 8th day are seen to stand much higher than in Urodela, still higher in the jumping sets. A comparison of the second transverse row of column I with the fifth transverse row of column III, shows in the other respect a strong influence of temperature; for it is easily seen that the former case which shows the development of 4 days in the medium of 0°C, stands very much lower than the latter case which represents likewise a development of 4 days, but in the medium of much higher temperature, e.g. 10°C. The results from the ascending series between 0°C and 10°C (*Table XV*), and those of 0°C and 5°C (*Table XVI*) confirm perfectly the assumption above given as regards the influence of temperature.

Table XV.

	I.	II.	III.
Time elapsed \ Temp.	0°C	5°C	10°C
Every 8/3 days	4	6	7
Every 4 days	6(−)	—	11(−)
Every 4 days		7	13

Table XVI.

	I.	II.
Time elapsed \ Temp.	0°C	5°C
Every 4 days	6(−)	8

In *Table XV* we see, besides the efficacy of the time consumed, a prominent influence of temperature, and a comparison of the present case with the corresponding case in Urodela (*Table V*) shows, how great the liability of Anura to temperature is. In the case from 0°C to 5°C (*Table XVI*) the thermal influence is not so strongly expressed as that just referred to, because the range of temperature was not so great; yet the thermal influence and a strong liability of Anura to it are incontestable.

b. Descending Series.

The set of eggs in this series have been arranged exactly in the same ways as in the corresponding cases of *Hynobius*, being divided into three cases. The results from the first case are represented in *Table XVII.*

Table XVII.

	I.	II.	III.	IV.
Time elapsed \ Temp.	15°C	10°C	5°C	0°C
Every 2 days	11	14	15	16
Every 4 days	16	—	—	17(−)
Every 8/3 days		9(+)	10(−)	10
Every 4 days		11	—	11(+)
Every 4 days			7	8(−)

Setting aside a striking advancement in stages, which is self-evidently due to the thermal liability of the Anuran eggs, there are obviously in this case the same factors as seen in Urodela: firstly, the development went on with the course of time, while the temperature grew lower by grades, (see the first, third and fifth transverse rows of *Table XIV* and the corresponding rows of *Table VII*); secondly, the *progress was practically given up, when the eggs suffered from a sudden change from a higher temperature to a lower* (see the second and fourth transverse rows of *Table XVII* and the corresponding rows of *Table VII*); the eggs passed into the dormant state brought about by the maximum negative efficacy of the thermal influence.

The tendency liable to lower temperature is, in the second and third cases (*Table XVIII*), expressed in a similar way as in the case just referred to (*Table XVII*). The only noticeable fact is that a progress in development is obvious in the lower temperature into which the objects were brought over suddenly from the higher temperature, as seen in the second transverse row of *Table XVIII* in contrast to the first named case (*Table XVII*); this is, as it is self-evident, because the lowest temperature, in which the development went on, was not 0°C which is hardly efficient in this respect,[1] but an efficacious grade of temperature, e.g. 5°C.

Table XVIII.

Temp. / Time elapsed	15°C	10°C	5°C
Every 8/3 days	11(+)	16	16(+)
Every 4 days	16	—	17
Every 4 days		11	12

1) Inefficiency of this temperature upon the Amphibian development is seen in the second and fourth transverse rows of *Table VII*, those of *Table XVII*, in columns I and II of *Table XIX* and in columns II and III of *Table XX*.

c. Reverting Series.

There are two cases distinguished, in one of which the intermediate temperature is lower than the first and final temperatures, while in the other this is higher than the two others. As shown in *Table XIX*, the influence of lower temperature is, in the reverting series 15°–10°–15°, *entirely covered by that of higher ones*, that is to say, the oldest stage arrived at in this case is equal to that of the development throughout 8 days in the higher temperature (compare with column I of *Table XII*). Only in the next series (series of 15°–5°–15°), the influence of lower temperature becomes visible: the oldest stage of this series corresponds to that of 5th day of the eggs exposed unbroken in the higher temperature, 15°C, while in the series in which the intermediate temperature was represented by 0°C, the lowered temperature did not turn its influence markedly. In the series of 10°–5°–10°, the influence of the lower temperature is, on the other hand, quite conspicuous, the final stage being equal to that of the eggs, which have developed 7 days in 10°C. Lastly, in the series of 10°–0°–10° the oldest stage attained only the 6th day of the development in 10°C.

Table XIX.

Every 8/3 days	15°C	10°C	15°C
	11(+)	16	19
	15°C	5°C	15°C
	11(+)	12(+)	17
	15°C	0°C	15°C
	11(+)	11(+)	16
	10°C	5°C	10°C
	9(+)	11(+)	14
	10°C	0°C	10°C
	9(+)	9(+)	12

The results of the second class, in which the intermediate temperature is higher than the two others, is represented in *Table XX*.

Table XX.

Every 8/3 days	0°C	15°C	0°C
	4	13(+)	14(−)
	0°C	10°C	0°C
	4	9	9(+)
	5°C	10°C	5°C
	6(+)	10	12(+)
	10°C	15°C	10°C
	9	15	17(+)

In the first series (series of 0°–15°–0°), the oldest stage attained hardly the stage of 3rd day of the eggs exposed continually in the higher temperature, 15°C, and in the second series (series of 0°–10°–0°), that of 3rd day of the eggs developed likewise throughout in the higher temperature, 10°C. In the series of 5°–10°–5° and that of 10°–15°–10°, the most advanced stage falls, in either case, into the stage of the 2nd and 6th day of the eggs exposed in respective higher grades, e.g. 10°C and 15°C.

In the two reverting classes, it is very obvious that the influence of the temperature is expressed in a remarkable manner; the temperature in which the eggs sojourned longer, affected the progress of development more. In the *first class* (*Table XIX*), the development advanced at least as far as up to the stage of the 8th day of the eggs reared in the higher temperature and shows that the lower temperature had no influence upon the progress at all, as seen in the first series of the class in question, because the higher one was here in prevalence. *In the second class where the lower temperature was in prevalence, the development attained only the stage of the 2nd or 3rd day of the eggs exposed throughout in the higher temperature.* There is only a series which advanced up to the stage of the 6th day of the eggs developed exclusively in the higher temperature; this is only because the lower temperature itself was represented already by a much higher one.

In short, what we have confirmed in Urodela proves to be exactly true to Anura. The only difference between the two forms consists in further advancement of the ultimate stage in the frog than in the newt, and this is owing to nothing other than a strong liability of the former to thermal influence.

III. Historical Review and Concluding Remarks.

So far as concerns the fact made out in the present observation, the development stands under control of the time it spends and of the temperature in which it goes on. Under a *fixed temperature,* in which the duration of time represents the only factor, the development is ruled by the time spent. But this is the case only with the higher temperature selected in the present experiment (see column I and II, *Table III* and *XII*); at the lower temperature (column III and IV, *Table III* and *XII*) a progress of development is pronounced scarcely (*Table III*) or not at all (*Table XII*). It follows that the *time consumed has little or no efficacy upon the development, when the temperature is very low. Not only this, but the action of lower temperature went so further that the physiological function of the eggs was totally destroyed by it, as stated above in the case of Rana fusca which developed in the medium of 0° C.*

Similar facts have been made out by O. Hertwig (1894) and O. Schultze (1895). According to Hertwig, at 0°C into which the eggs were carried over soon after the fertilization, the cleavage did not appear in the course of 24 hours; the eggs suffered, moreover, from the low temperature so severely that they developed only so much further as to be divided into blastomeres and to cause injury in the yolk-hemisphere, when they were transferred in the medium of normal temperature. Schultze carried out the experiments with the eggs of 3 different stages: the youngest lot represented by the eggs with the dorsal blastoporic lip just appearing, the second lot represented by the stage at the end of the gastrulation and the oldest lot of embryos in which the medullary folds were closed. After a sojourn of 3 days in the medium of 0°C they were found to show no sign of development; when they were taken out into a normal thermal medium, the development went on as usual. In the case in which the sojourn in the cold medium had been prolonged into 2 weeks, the result was the same as that just stated, that is to say, the passage of time flew in vain. After transferrence into a normal thermal medium the development went on in the two younger lots, while the oldest eggs are reported to have fallen.

On the contrary, the results of the present experiments are not in agreement with those by KROGH (1914). Even in the temperature on which his curves are based (7°C–20°C) his affirmation can not be confirmed by my own results. As seen from the statement given above, every grade of temperature shows its own curve derived from the resultant with a certain duration of time. I confess, the scales of stage, which I set forth, are of course to a certain extent arbitrary, inasmuch as they may not be subserved as the standards to be compared exactly; they satisfy, on the other hand, what we want as the marks by which several cases of development are brought into comparison. The duration of time upon which KROGH's curves are based, may likewise be looked upon to some extent as an arbitrary one, because the time consumed for the preparation of the first cleavage or for the closure of the medullary groove varies to some extent, although these were examined by KROGH as the third element of his curves.

At any rate, according as the grades of temperature are fixed, the duration of time throws its influence upon the development, which varies in a certain range. At the lower temperature *it brings forth the negative efficacy on the vital activity on which the development depends, until the latter is destroyed by that temperature.*

Now, let us turn to the cases of *fixed intervals of time* in which the development went on, with the media of temperature which were changed. From the foregoing description 2 cases are recognized: the cases of the reverting series and those of the ascending and descending series. In the former we have pointed out that *the intermediate temperature representing the shorter interval of time, whether higher or lower, has little or no efficacy upon the development (Table IX, X, XIX, XX), which is accordingly regulated chiefly by the temperature of longer intervals* by which the experiments were commenced and closed.

In the latter (*Tables IV—VIII, Tables XIV—XVIII*), *the influence of time is fairly expressed in the development, when the temperature was changed step by step*; the same is the case, when the temperature was suddenly changed from the lower to the higher. On the contrary, *no efficacy is recognized upon the development, when the change of temperature jumped from the higher to the lowest.* In addition to the change of temperature a factor is thus represented by the mode, in which this change took place: whether *gradual or sudden.*

The *liability to the temperature* is obviously much greater in the frog than in the salamander and may probably be looked upon as a character acquired from the terrestrial habit of Anura. In the frog the 2 lots of eggs, which developed respectively in the medium of 5°C and in that of 0°C, are distinguished sharply from the other 2 lots reared at 15°C and 10°C on the stages arrived at in the close of 8th day (*Table XII*). While in the latter group the development has obviously advanced with the time spent, *in the former the advancement is scarcely recognized in the course of the latter 4 days*; and *this is particularly obvious in the lots developed under the influence of 0° C.* From these data it may be assumed that the *diminishing efficacy of the thermal influence commences its work at 5° C and attains its climax at 0° C, in combination with the course of 4 days; further course of time has in this medium of temperature no effect upon the development,* until the eggs are found to be dead at the close of 8th day, in which the experiment is closed.

It is, therefore, seen that the temperature upward to 5°C is favorable for early development of the frog, and that the temperature lower than this, for instanse, 0°C does not allow the eggs to develop longer than 4 days and to live longer than 8 days.

A glance upon *Table III* shows that Urodela has a tendency parallel with what is just stated of Anura; the former is distinguished from the latter only in thermal influence expressed so markedly. The salamander is shown to be capable, in the medium of 0°C, of development longer than 8 days.

The following table (*Table XXI*) is a compilation of April temperature of Sapporo in 10 years past (1912—1921).

Table XXI.

Air temperature in April, (1912—1921).

	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921
1st	−1.66	4.93	1.22	−0.76	0.74	5.71	2.08	−3.70	4.53	6.39
2nd	−0.43	1.37	−1.93	0.80	−0.24	2.30	2.42	−2.15	5.70	7.31
3rd	0.26	1.65	−2.97	3.07	0.33	4.63	1.51	1.00	5.51	4.65
4th	−0.30	3.66	0.03	3.42	−1.33	3.91	0.93	1.68	7.42	3.78
5th	−1.33	4.85	0.92	3.30	−2.08	1.13	2.34	4.26	7.63	3.41
6th	−0.39	6.55	2.05	2.77	−2.54	3.15	2.45	2.49	4.34	0.45
7th	2.14	6.00	4.71	2.44	−1.02	6.07	2.67	3.61	3.05	*1.05*
8th	2.26	3.16	4.56	2.13	−0.74	2.99	1.93	4.61	2.67	1.73
9th	4.97	1.30	4.41	5.15	1.54	5.19	2.32	2.78	4.16	3.84
10th	4.01	2.17	4.18	2.07	4.73	6.07	3.85	2.82	4.20	*6.53*
11th	0.72	3.84	2.27	2.15	7.11	4.61	3.04	5.13	6.03	6.85
12th	3.87	7.20	2.51	5.19	7.72	3.46	5.25	2.03	7.67	6.72
13th	11.47	4.09	3.47	5.93	2.45	3.69	5.95	4.70	8.45	8.24
14th	11.53	5.71	1.09	2.70	2.23	5.12	6.49	8.17	6.95	7.88
15th	9.54	5.42	2.58	4.72	5.13	5.69	6.08	7.65	5.59	6.90
16th	3.72	4.24	1.42	7.86	4.70	7.39	8.28	4.70	6.56	4.33
17th	2.59	0.65	2.30	5.10	5.73	3.24	7.58	4.55	10.25	5.81
18th	3.92	2.22	5.35	4.37	7.01	2.05	8.94	4.58	9.34	4.40
19th	5.77	5.51	11.95	4.94	8.52	2.72	6.91	7.63	9.58	3.88
20th	6.18	7.89	9.15	2.06	7.24	4.16	6.84	5.04	7.49	7.66
21st	7.35	6.18	8.85	1.35	5.72	3.57	5.02	7.91	6.52	*9.29*
22nd	7.54	8.69	8.71	2.66	3.90	6.87	4.75	8.98	5.62	*10.43*
23rd	7.89	10.30	3.12	1.87	5.10	6.98	3.18	8.45	2.93	11.63
24th	6.48	9.46	7.62	2.40	6.67	4.01	4.29	7.68	2.53	7.43
25th	7.63	7.66	11.87	4.67	4.02	5.46	6.60	9.38	5.45	9.75
26th	2.35	7.71	11.33	6.59	9.24	9.24	6.61	11.07	6.95	12.06
27th	5.24	6.53	9.14	9.71	3.90	11.78	6.64	5.64	7.62	12.38
28th	8.19	11.02	6.72	8.60	6.71	8.00	8.27	4.49	8.90	9.93
29th	10.27	11.18	6.60	8.71	9.63	2.50	9.49	7.20	7.64	10.53
30th	11.77	10.68	8.73	3.38	10.06	3.63	11.25	8.05	9.06	10.43

The spawing was in April last extended in the salamander between the two dates represented by italics and in the frog between those shown by heavy italics.

We see that with a few exceptions of minus grade, the temperature under influence of which the Amphibian eggs develop, ranges from 0°C to 12°C. In some two weeks occupied by the spawning of the animals, the temperature shows also a similar range of changes, to be raised gradually afterwards. It is noteworthy that a severely cold weather did not ever extend itself over 2 days, so that the eggs are not influenced much by a low injurious temperature. The development in field is in this way favoured generally; a case I watched in a small pool in the university garden is given as follows (*Hynobius*).

Table XXII.

date (Apr.)	19th	20th	21st	22nd	23rd	24th	25th	26th
stage	5(−)	7	8(−)	9(+)	12(−)	14	16(−)	16(+)

In spite of a low temperature, in which the eggs were reared, early development was not markedly prevented, as is very obvious in all the tables given. The further the stages proceeded, the more the progress of development was resisted by low temperatures. It seems to be probable, that in early stages the eggs are stronger than in advanced stages in resisting the low injurious thermal influence, because in the former case the whole development consists simply of cell-divisions, whereas in later stages a large amount of vital activity is required to be employed at least in the progress of folding. In nature this demand is approximately fulfilled in favour of the development, being likely afforded by the temperature rising towards later stages (*see Table XXI*).

Sapporo, July, 1921.

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Explanation of Figures.

a list of the abbreviations.

a. anal-pit.
b. blastopore.
e. eye.
eg. external gill.
f. invaginated furrow of blastopore.
gr. gill-ridge.
gs. gill-slit.
h. head protuberance.
ha. hyoid arch.
m. medullary fold.
ma. mandibular arch.
ms. mesodermic somite.
n. neural groove or dorsal furrow (Rückenrinne).
s. stomodaeal depression.
sk. sucker.
t. tail-bud.
v. visceral arch.
y. yolk-plug.

Plate I. (*Hynobius*).

Fig. 1. Stage of advanced gastrulation (Stage 9).

Fig. 2. Stage of old gastrula (Stage 11).

Fig. 3, a, b. Stage in which medullary folds just appeared; *a.* anterodorsal view; *b.* posterodorsal view (Stage 12).

Fig. 4, a, b. Stage with medullary folds distinct throughout their entire extent; *a.* anterodorsal view; *b.* posterodorsal view (Stage 13).

Fig. 5, a, b, c. Stage with medullary folds brought close to each other; *a.* front view; *b.* dorsal view; *c.* posterodorsal view (Stage 14).

Fig. 6, a, b, c. Stage with fusing medullary folds and 4 pairs of mesodermic somites; *a.* front view; *b.* dorsal view; *c.* postrodorsal view (Stage 15).

Fig. 7, a, b. Stage with head protuberance, distinct anal-pit, 6 pairs of mesodermic somites and 3 pairs of gill-slits; *a.* ventrolateral view; *b.* dorsolateral view; (Stage 16).

Fig. 8. Stage with eyes, 12 pairs of mesodermic somites, 4 pairs of gill-slits, anus and tail-bud furnished with caudal fin (Stage 17).

Fig. 9. Stage older than that represented in *Fig. 8*, with eyes, many pairs of mesodermic somites, stomodaeal depression, mandibular, hyoid and visceral arches, anus and distict tail (Stage 19).

Plate II. (*Rana*).

Fig. 10. Stage of advanced gastrulation (Stage 9).

Fig. 11. Stage of old gastrula (Stage 10).

Fig. 12. Stage of gastrula with dorsal furrow just appeared (Stage 11).

Fig. 13. Stage in which medullary folds just established (Stage 12).

Fig. 14. Stage with gill ridges and medullary folds strongly raised and brought close to each other in their posterior extent (Stage 13).

Fig. 15. Stage with medullary folds brought close to each other, until their cephalic extremities (Stage 14).

Fig. 16, a, b. Stage with completely fused medullary folds and 3 pairs of gill arches and sense plate; *a.* dorsal view; *b.* lateral view (Stage 15).

Fig. 17. Stage with a pair of suckers, 3 pairs of gill-slits and raised tail-bud (Stage 16).

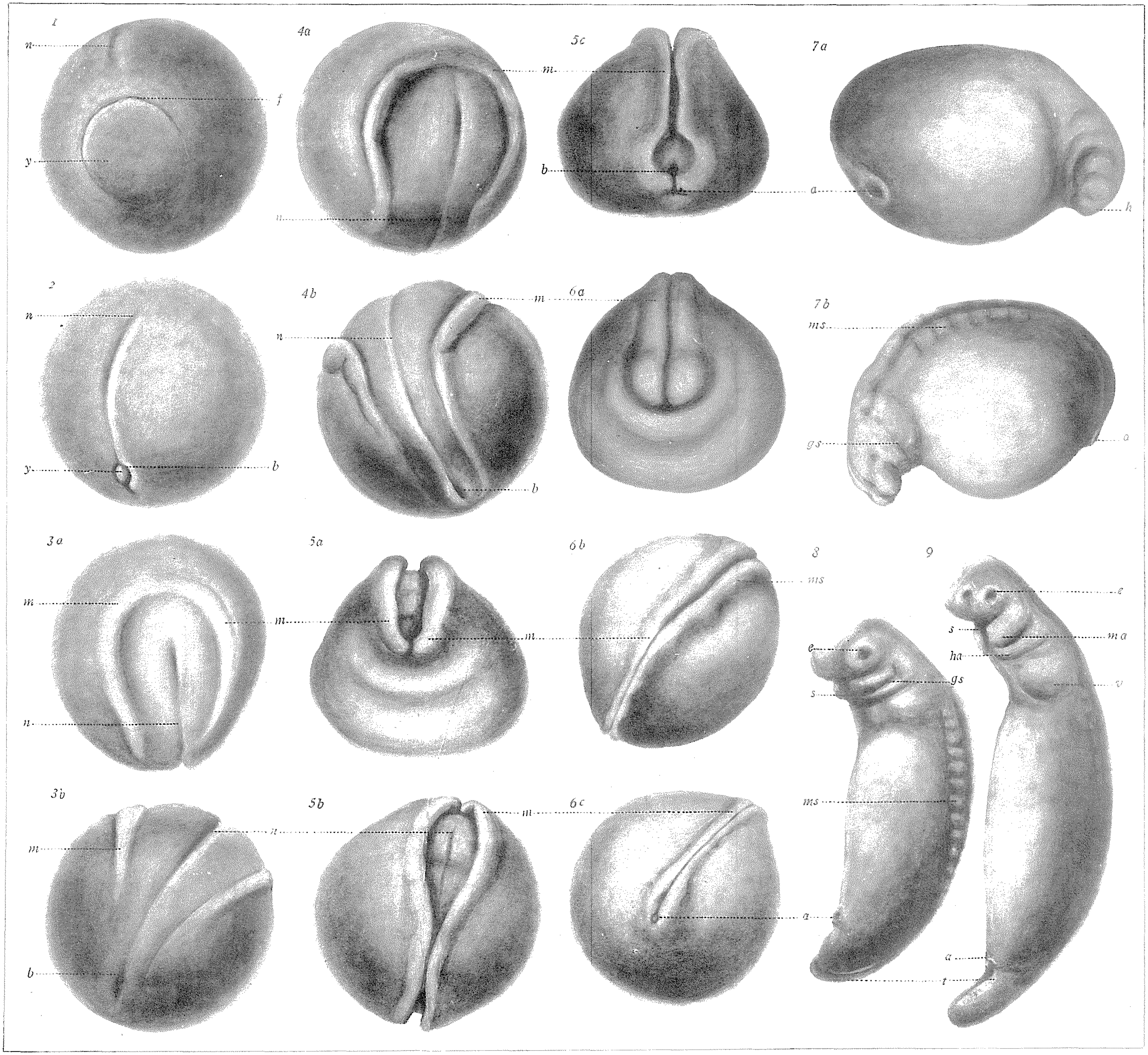
Fig. 18, a, b, c. Stage with eyes, about 13 pairs of mesodermic somites and tail-bud furnished with dorsal and caudal fin-folds; *a.* dorsal view; *b.* ventral view; *c.* lateral view (Stage 17).

Fig. 19. Stage with external gills, many pairs of mesodermic somites and distinct anus (Stage 18).

Fig. 20. Stage with stomodaeal depression, suckers, tail and strongly developed external gills (Stage 19).

Fig. 21. Older stage from the same lot as one represented in *Fig. 20* (Stage 19).

Pl. I.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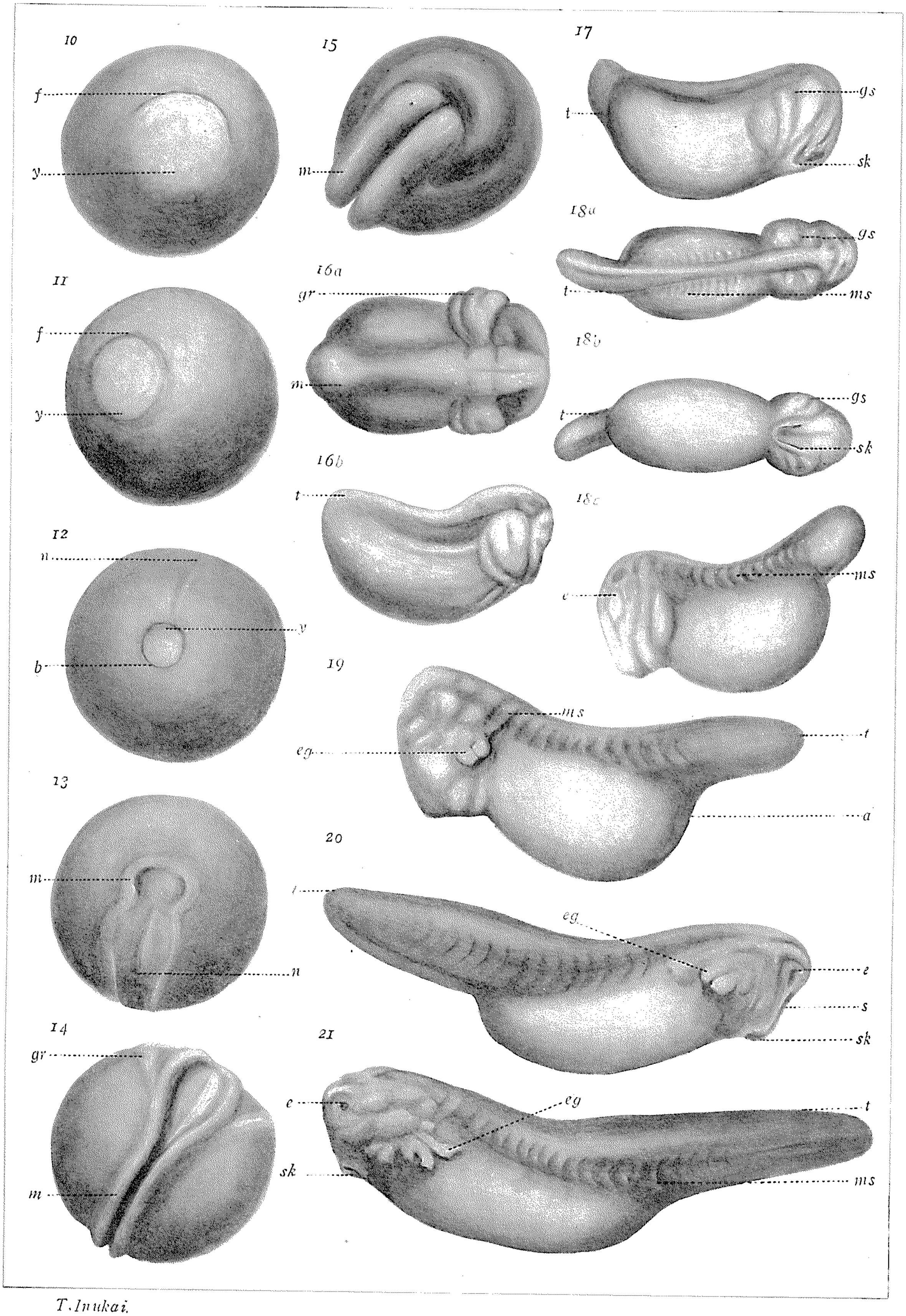
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