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**On a Japanese Salamander, in Lake Kuttarush, which  
Propagates like the Axolotl.**

By

**Madoka Sasaki.**

From the Hokkaido Imperial University, Sapporo.

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With Plates I-III and Textfigures 1 & 2.

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As is familiar to students of the subject, the Axolotl had been looked upon as a species of Perennibranchiates under the generic name *Siredon*, until it was made known by Duméril (1867), from observations in the Jardin des Plantes at Paris (Meyer 1865, 1866, Duméril l. c.), to be the larval form of a terrestrial salamander, *Amblystoma tigrinum*. This unexpected discovery caused zoölogists to turn their attention to this form of the species, so that experimental and anatomical as well as embryological works regarding it succeeded thereupon one after another. Among the authors who occupied themselves with these subjects the following may be named: Kölliker, Weismann, von Chauvin, Schubert, Camerano, Barfurth, Metzdorff, Wolterstorff, Siebold, Gadow, Gudernatch, Adler, Uhlenhuth, Kaufman, Swingle, etc. Their efforts consisted chiefly in making clear what is efficacious in leading the same species to propagate in two ways: (1) as a larval form, e. g. the gill-breathing Axolotl and (2) as an adult form, e. g. the terrestrial *A. tigrinum*, breathing with lungs.

A parallel case occurs in our country, in the *Hynobius lichenatus* Boulenger, a salamander which is abundant in Hokkaido, and which closely resembles the above mentioned Axolotl, in several respects, especially in reproduction. More than ten years ago I found this Urodelan in a mountain lake where it abounds in immense numbers, and took advantage of this to closely observe its actual spawning. Part of the neotenuous individuals collected there I have now in my possession as preserved specimens, but the majority I kept in aquaria of my laboratory for ex-

periments. My further attention was drawn to the transformed mature form of the salamander, inasmuch as little is known about it, and efforts have been made to gather such data relative to its life history as might be of interest.

The present paper embodies the results so far obtained and deals chiefly with an outline survey of bionomics of *Hynobius lichenatus*, extending to the origin of neoteny owing to the physical conditions of the lake known as "Kuttar-ushi,"\* not far from the Hot Spring of Noboribetsu.

### Taxonomy

Even the taxonomic characteristics of the species have not as yet been fully made out. The type specimen which I believe to be a juvenile just having finished its metamorphosis, is the single individual of the species hitherto fully described. The original description of the species (Boulenger 1883) has been little added to by Stejneger (1907) and by Tago (1907).\*\* It is admirable that with this just transformed specimen the identification was so correctly made. I have re-examined the species characters, employing some hundreds of full-mature specimens at my disposal which are mostly more than twice the type in measurement. From the results of this revision the following taxonomic diagnosis is derived.

The vomero-palatine teeth are about 25 in number and form an uniserial band as usual. The band forms the shape of a laterally expanded M, the depth of which is equal to, or less than, one-third the breadth; the median posterior angle exists just on the line connecting the posterior extremities of both the outer branches or extends, though very little, beyond it. The posterior extremities are situated just behind the centres of the choanae, and the anterior angles of the M extend a little forwards beyond a line passing through the anterior edges of the choanae. So delineated, the dentition is quite peculiar among the *Hynobius* hitherto known, as has been pointed out by Stejneger (l. c.). But such a case as occurred in the type—in which the band is said to have the posterior median angle not extending posteriorly beyond the extremity of the outer branches—is very rare in the specimens at my disposal.

\* *Kuttar-ushi* is a Ainu dialect, and means "the place of *Polygonum*"; or it may mean, if applied to the lake, "Hollow-lake" (after Dr. J. Batchelor).

\*\* Hashimoto (1910) notes mature specimens, but no description of specific characters is given.

The head is of moderate size. Its length measured from the gular fold anteriorly is just, or even a little shorter than, one-third that of the body, which length is taken from the above fold caudad to the anterior margin of the cloacal opening; a length decidedly less than that given by Boulenger (l. c.). The shape of the head of the adult varies in different sexes. In the female it is roughly oval in outline, with a blunt snout (Pl. I, fig. 2.). This is also the case in the young stage of both the sexes, agreeing with the illustration of the type. But in the mature male, the outline rather approaches to triangle, the large temporal muscles giving the head a swollen contour behind (Pl. I, fig. 1.). It is broader, and more depressed in the male, than in the female. In short, the head of the male is of robust build, as compared with that of the female.

The eyes are rather large and prominent, as originally described; the longitudinal diameter is about one-third the breadth of the head. The gular fold is strongly marked around, and is deepest underneath. A distinct transverse groove runs upwards on each side of the head, just behind the angle of the jaw, and divides itself into two branches, of which the anterior one proceeds forwards to join the hind margin of the eye, while the posterior runs to the gular fold.

The body is of moderate size, and proportioned like that of *Hynobius nigrescens*. The costal grooves are 11 in number, which is also the case in the type specimen. In this characteristic, the species is again allied to *H. nigrescens*.

The fore and hind limbs are of about equal length, and about one-third the length of the head and body taken together. As to the thickness, the hind limb much exceeds the fore limb, especially in the male, in which the fore limb is about twice as thick as the hind. The fifth toe is, though the thinnest of all, by no means rudimentary as described and illustrated by Boulenger (l. c.). Its length is about that of the first toe, or frequently, even a little longer, and measures  $1/3-1/4$  the length of the third toe, which is the longest of all.

The tail is long, and its length is about that of the head and body taken together. It is strongly compressed, and keeled above and below, the upper keel originates some distance behind the posterior end of the cloacal opening, and the lower keel, some distance behind the origin of the upper keel. The tip is cuspidate.

The colour of a young, recently metamorphosed, is just like that of the type, having a lichen-like deep grayish variegation in preserved state, whence the specific name "*lichenatus*" is derived. But that of mature indi-

viduals is quite different. The ground colour of the dorsal and lateral surfaces is uniformly a deep purplish brown, of which the intensity varies in some degrees, in different individuals. Microscopical minute greenish dots of metallic lustre may appear on the dorsal and lateral surface. The colour underneath is pale.

The cloacal opening differs in structure according to the sex. While in the female it is only a longitudinal slit, that of the male is an Y-shaped opening, with a papillary protuberance at each of the three angles, that of the anterior median angle being most prominent and most well defined. The posterior extension of the opening is connected posteriorly with a caudal groove which runs from the opening for some distance backwards along the ventral surface of the tail.

The present species is among *Hynobius* nearest to *H. nigrescens* in general structure in costal grooves and in coloration. This taxonomic relationship is supported further by other biological characteristic, as mentioned later.

### Distribution

The present species is the only Urodele Amphibian in Hokkaido, as listed by Hatta (1910, 1913, 1921). Tago (1907) reports *H. nigrescens* (his *fuscus*) from Ishikari of Hokkaido, but I cannot confirm this. Hashimoto (1910) likewise lists this species, erroneously, among the Hokkaido Urodelan fauna, basing his conclusion upon the specimens preserved in our university. The absence of *H. nebulosus* from Hokkaido needs no explanation as pointed out by Stejneger (1907).

The recorded localities of *H. lichenatus* are: Aomori, Honshu (type); Shiraishi and Maruyama, Ishikari (Hatta and others, 1910); Chitose (Hatta and others l. c.); the two latter localities are in Hokkaido. The specimens now before me were collected from the following localities: near Onuma, Oshima; Osawa, Shiribeshi; Makkarinupuri; Jozankei, Ishikari; Mt. Moiwa; Sapporo; Noboribetsu, Iburi; Teshiwo; and Hidaka. According to my friend, Mr. H. Tanakadate, a geologist who has travelled all over Hokkaido, the species also occurs, in the following places: Kokkuri-ko; Iwaunupuri and its vicinity, all in Shiribeshi; river-system near Toya-ko; Shikaribetsu-numa; Akan-ko; Kutchara-ko; and Mash-ko. The localities enumerated, therefore, cover nearly the whole of Hokkaido, and the northern extremity of Honshu. From the zoogeographical viewpoint, its occurrence on both sides of the Tsugaru Straits is of interest,

inasmuch as they have nothing to do with such a lower Vertebrate as the form in question, as pointed out by Hatta (1913). This fact invalidates the view of a zoogeographical separation called the Blackiston's Line (Blackiston 1883).

*H. lichenatus* marks the northern limit of distribution of the genus. It is interesting that *H. nigrescens* which is very close to *H. lichenatus* in its taxonomic characters, is found in northern parts of Honshu: Sendai (type); near Sendai (Tago 1907); Nikko (Stejneger 1907, Tago 1907); Mt. Tokakushi, Shinano (Tago 1907).

The altitudinal distribution ranges high up the summit of high mountains. The specimens in my possession have been collected from every level of them above the sea. The positions found by Tanakadate are ca. 750 m. (Kokkuri-ko), ca. 800 m. (Shikaribetsu-numa), and ca. 900 m. (Iwainupuri) above the sea level. Some of my students have often brought to me fresh spawns from transient pools near the summit of Mt. Teine (980 m.), not far from Sapporo. The salamander is further conspicuous, because it represents Vertebrates in association with some salmonoid fishes in comparatively fresh volcanic lakes of Hokkaido.

### Size of Adult

The size of the biggest male I met with, was enormous, measuring 200 mm. in total length. But I have never seen such a rare one since. About 600 mature specimens which I collected on April 12th, 1924, range, in male, 146-185 mm. in total length, and in female, 137-166 mm.; the mean size in both sexes is the commonest. As a whole, the female is a little smaller and more slender than the male.

### Habitat

As is well known, Urodelans show every graduation in occurrence from aquatic to terrestrial, but fall in the main into the following three categories: In the first place, thoroughly aquatic in life, even after metamorphosis, as occurs in *Cryptobranchus* (Sasaki Ch. 1887, Ishikawa 1904, Smith B. G. 1907, 1912); secondly, terrestrial even at the time of birth, as represented by *Salamandra atra* (von Chauvin 1877, Nnoblau 1904, Kammerer 1904); lastly, sometimes aquatic and sometimes terrestrial, which is the commonest mode of life in Urodelans. In the last named mode there is a great variation, of which the commonest

type is such that the organism is reared in the water, passing the larval life in the same medium; after metamorphosis, however, it becomes quite terrestrial except at the breeding period when it again inhabits the water.

Now, the life of *Hynobius* to which the present species belongs, may also be included in the last category. But *H. lichenatus* is peculiar in visiting the water in adult form, even at periods other than the breeding season, as seen from the collections which I made several times from mountain pools near Sapporo; viz. on Sept. 2nd, 1918; Aug. 25th, 1920; and Oct. 19th, 1922.

The habitat of the species on land is shady damp places, on mountain slopes, or in forests or in their vicinities. This is also the case in other *Hynobius* as mentioned by Yamada (1903), Tago (1907) and Kunitomo (1910). The creature is to be found during the daytime in these places, always concealing itself under rotten leaves or fallen trees, usually in the vicinity of water, but it may be found separated sometimes, two miles or even more, from any kind of water. It prefers pools, with clear water, and often with decaying leaves and branches deposited on the bottom. In such waters the individuals are usually found resting quietly on the bottom, being sheltered by various objects, and come up at times to the surface for respiration.

In captivity it sought crevices under stones or an angle of the vessel in which it was kept; a habit which Smith B. C. (1907) mentions in *Cryptobranchus alleghaniensis* as a thigmotaxis.

At night it is active and leaves its shelter in search of food which consists of various living worms as will be mentioned later. From this, it follows, that the present species is nocturnal in its habits as in most other Urodelans hitherto observed (Ritter & Miller 1899, Eycleshymer 1906, Smith B. G. 1907, Tago 1907, Piesol 1909, Gadow 1909).

### Feeding Habit

As described above, the species subsists on living worms. The stomach contents I examined, consisted of the adults and larvae of insects, freshwater crustaceans, centipedes, earthworms, and occasionally even small fishes. Similar prey have been found in the stomachs of other *Hynobius* (Tago 1907). This predaceous habit agrees with all literature treating the habits of Urodelans in general. They are voracious especially in the larval stage.

In captivity it always occurred, that the captives seized their prey

with the mouth, turning the head with great quickness when the prey was brought within a very few centimeters of the mouth. A similar habit is also reported by Reese (1903, 1905), and by Smith B.G. (1907), both in *Cryptobranchus alleghaniensis*. In seizing the prey the tongue is of little use. It lies almost flat on the floor of the buccal cavity, and is not capable of protruding as in the case of *Torius*, *Spelerpes*, and *Chioglossa*. This is also true of other *Hynobius*, as far as I have been able to observe. When hungry in captivity, the creatures always ventured to assault any mate which happened to be within easy reach of their mouth. This habit is especially noticeable in the larval stage, in which the nibbling of each other's gill and tail is a matter of very usual occurrence even with a sufficient food supply. Yet their great tenacity of life has been very frequently commented on. In my aquaria many have been kept alive for months without food.

### Locomotion of the Adult

The locomotion of Urodelans in general is of special interest in view of the fact, that it represents in every particular the primary mode of Vertebrates' walking on the land. The mode is described by Smith B.G. (1907) in *Cryptobranchus alleghaniensis*. Eycleshymer (1906) mentions that of *Necturus maculosus*. The mode of *Autodax* is reported by van Denburgh (1895) and by Ritter and Miller (1899). As a general rule, in this group, the limbs give little support to the body, so that the abdomen lies in contact with the ground, and the locomotion is carried on by a crawling action of the limbs. At each action of the limbs the body curves to right and left alternately, counteracting the imperfect articulation of the limbs to the body. This is the reason why the movement is generally very slow and inactive; and on the lateral flexibility of the trunk lies the physiological significance of the costal folds. Correlated to these, the musculature and skeleton show very primitive condition as already mentioned by Maurer (1892, 1905) and by Osawa (1901). The walking mode which I repeatedly observed on the present species differed in no way from that given above. In each movement, the diagonally opposite limbs act in union, and while the forward stretching of such a pair is taking place, the remaining pair resist against the ground, which effects to shift the body forwards. The proximal half of the limbs is so fixed to the body that it is not capable of swinging forwards much beyond the right-angle position to the body axis; accordingly, in each stretching of the limbs

the body must curve to either side as mentioned above. The tail assists the shifting of the body to some extent curving sideways and in a direction opposite to that of the body. Beyond this, however, it seems to have no use, showing no marked prehensile power as in *Plethodon cinereus* (Piersol 1909) and *Autodax* (van Denburgh 1895, Ritter and Miller 1899), nor acting as an organ of leaping as in the latter genus (l. c.), nor showing any autotomy as is known in *Plethodon cinereus* (l. c.), and in *P. oregonensis* (Hubbard 1903).

In swimming, the present species exhibits the mode of ordinary fishes. Progress made by an undulatory movement of the body along a horizontal plane, the tail also sharing in the motion. But the most rapid swimming is chiefly produced by the vigorous lateral propulsion of the tail. While swimming, the limbs are of little function except to preserve the equilibrium of the body. They are held back close to the body in such a position as to lessen the resistance of water, to which they are subjected during the swimming. The swimming never covers a long distance; at most a few meters. Then the creature seeks concealment beneath an object. A similar habit is also given by Eycleshymer (1906) in *Necturus maculosus*, by Smith B.G. (1907) in *Cryptobranchus alleghanensis*, by Gadow (1909) in *Spelerpes porphyriticus*.

### Ecdysis

The moulting of the epidermis has often been noticed in various species of Urodelans. Fischer (1886) describes it in *Salamandrina perspicillata*, Kneeland (1857) in *Necturus*, Ritter (1897) in *Molge torosa*, Tago (1907) in *Molge pyrrhogaster*, Smith B.G. (1907) in *Molge viridescens*, and Yamada (1903) in *Hynobius nebulosus*. According to most of these descriptions the raised epidermis of the body is flayed off gradually from the mouth backwards, and that of the limbs, from proximal to distal, like the fingers of a glove turned inside out. The same thing also occurs in the present species, and the mouth is sometimes used to aid in the removal of the fragments. The habit takes place most frequently in younger stages. The shed epidermis may be eaten by the creature which casts it off, as has occasionally been observed in many other forms, but in the present species it seems by no means to be any important article of diet, thus disproving Ritter's assumption regarding this subject for *Molge torosa* (l. c.).

### Sexual Dimorphism and Sexual Ratio

As mentioned before, the adult male and female can be distinguished by the structures of the head, cloacal opening, and hind limbs. But otherwise, the species exhibits no marked sexual dimorphism either in shape or in coloration, a feature probably correlated with the reproductive habit and which is a good contrast to that of markedly sexual-dimorphic forms like *Molge cristata*, in which a lengthy sexual courtship takes place. The gravid female is known not only by the marked swelling of the belly but also in having prominent margins of the cloacal opening. The male exhibits no special swelling around the opening even in full bloom.

The numerical ratio of the sexes has been about 1/1 as far as estimated from individuals reared from eggs in aquaria, as follows:—

| No. of complete spawn from a female which were reared for the experiment. | Number of males. | Number of females. | Number of individuals which died or were eaten by others before determination of sex. |
|---|------------------|--------------------|---|
| i   | 25               | 23                 | 14  |
| ii  | 31               | 35                 | 7   |
| iii   | 28               | 27                 | 12  |
| Total   | 84               | 85                 | 33  |

Another example of the sex ratio has been taken from among the full-grown individuals which I collected by thoroughly raking out mountain pools during a series of years, as follows:—

A. *Collections from pool 2 < 3m. at foot of Mt. Moirwa.*

| Date           | Male | Female | Total |
|----------------|------|--------|-------|
| 1914, April 20 | 63   | 90     | 72    |
| 1916, April 23 | 59   | 18     | 68    |
| 1920, April 16 | 54   | 21     | 75    |
| 1921, April 14 | 26   | 12     | 38    |

B. *Collections from pool about 4 × 6m. found among bushes near foot of Mt. Moirwa.*

|                |     |                  |     |
|----------------|-----|------------------|-----|
| 1921, April 21 | 346 | 58               | 402 |
| 1922, April 18 | 289 | 31               | 320 |
| 1923, April 16 | 459 | 81               | 548 |
| 1924, April 12 | 474 | 136 (gravid, 70) | 610 |

C. Collections from pool about 3-5m. at foot of Mt. Maruyama near Sapporo.

|                |    |    |    |
|----------------|----|----|----|
| 1920, April 14 | 36 | 22 | 58 |
| 1921, April 14 | 53 | 36 | 89 |

D. Collection from well 1m. cross and 1m. deep found near pool C.

|                |   |   |   |
|----------------|---|---|---|
| 1920, April 14 | 4 | 3 | 7 |
|----------------|---|---|---|

In all the above cases, the male is exceedingly superior in number to the female. The difference of the ratio from that given before is due to a special reproductive habit of the species. The male enters waters early in spring and remains there a long time, almost until the close of the season, whereas the female comes late to the waters, and when the spawning is over, soon leaves them or retires towards their margin to hide under various substances beyond reach of our hands; accordingly the females collected at a given time in pools are very few in number as compared with males. A similar habit is mentioned also in *Necturus* by Eycleshymer (1906) and in *Cryptobranchus alleghaniensis* by Smith B. G. (1907, 1912).

### Breeding Season

The present species takes a vernal breeding as in most other Urodelans, and the mating and spawning occur simultaneously in the breeding period. The period varies to some extent with the altitude, and when in the same altitude, with the climate of the year. In a low altitude, as in the vicinity of Sapporo, the season is April, while at high altitudes, as in mountains, it may be May or even late June. I have collected fresh spawns in May from pools and lakes near Noboribetsu; whereas the students' collections from the summit of Mt. Teine dated in June.

In other Urodelans, e. g. in *Hynobius nebulosus* (Kunitomo 1910) and in *Cryptobranchus* (Smith B. G. 1912) the climatic influence upon the spawning consists only in the atmospheric temperature, but here, in my case, it involves one more factor, i. e. the amount of snow; both the agencies are profoundly connected with the melting time of ice and snow which cover over the spawning pools during the winter. The following table shows how closely the atmospheric temperature and amount of snow of March and April are related to the date when the first spawn appears in the year in two pools of Mts. Moiwa and Maruyama.

| Year. | Date of first spawning in Moiwa pools. | Date of first spawning in Maruyama pools. | Mean atmos. temperature of March. | Mean atmos. temperature of April. | Depth of snow at the beginning of March. | Depth of snow at the beginning of April. |
|-------|--|---|-----------------------------------|-----------------------------------|--|--|
| 1914  | —                                      | 6th, April,                               | 1.2° C.                           | 4.8° C.                           | 14.7 cm.                                 | 0.0 cm.                                  |
| 1915  | —                                      | 10th „                                    | -2.2 „                            | 4.0 „                             | 48.2 „                                   | 2.6 „                                    |
| 1916  | 11th, April                            | 11th „                                    | -3.1 „                            | 4.1 „                             | 26.6 „                                   | 3.4 „                                    |
| 1917  | 9th „                                  | 8th „                                     | -1.4 „                            | 4.9 „                             | 68.4 „                                   | 1.0 „                                    |
| 1918  | 8th „                                  | 6th „                                     | -0.8 „                            | 5.2 „                             | 33.9 „                                   | 0.0 „                                    |
| 1919  | 9th „                                  | 7th „                                     | -1.1 „                            | 4.9 „                             | 26.5 „                                   | 0.0 „                                    |
| 1920  | 9th „                                  | 6th „                                     | -0.2 „                            | 6.4 „                             | 44.7 „                                   | 0.0 „                                    |
| 1921  | 9th „                                  | 8th „                                     | -1.7 „                            | 6.8 „                             | 50.9 „                                   | 1.2 „                                    |
| 1922  | 13th „                                 | 10th „                                    | -2.5 „                            | 6.4 „                             | 48.7 „                                   | 2.4 „                                    |
| 1923  | 10th „                                 | 8th „                                     | -0.3 „                            | 4.7 „                             | 67.3 „                                   | 0.2 „                                    |

As far as I have observed, the water temperature of the pools ranged 3°C - 5°C at the first appearance of spawns when some remains of snow were found here and there in the waters. As seen in the above table, the spawning began a few days earlier in the pools of Mt. Maruyama than in those of Mt. Moiwa, due to their difference in physical conditions: in the former pools which are sheltered from the sun-shine by fewer bushes and trees, ice melts some days earlier than in the latter.

The spawning period lasts two weeks or a little more, as pointed out by Inukai (1922), and the climax of the spawning does not fall exactly in the middle of the period, but is reached a little prior to it.

Unseasonable spawning takes place sometimes, especially in the fall, in pools with cold spring water. In the latter part of October I twice found fresh spawns in pools of Mt. Moiwa, the first time the water temperature showed 6°C, and the second time, 7°C. The gill-bearing larvae about three weeks old may also be found in early winter. Further, it is not an uncommon thing to find over-wintered small larvae together with the first spawn of the year in one and the same pool. Such an instance of the unseasonable spawning in the fall is also reported by Metzendorff in *Amblystoma tigrinum* (1896).

### Breeding Ground

The breeding ground is very like that of *Hynobius naevius* and *H. nigrescens* given by Tago (1907), consisting of pools or lakes, with clear,

cold, and quiet water which are situated on mountains or in forests or in their vicinities. Many I have met with, were surrounded by bushes and trees, with their bottoms covered with decaying leaves and branches; and many logs and fallen trees were also frequent adornments of the margins. Very often they were transitory pools of water formed by melted ice and totally dried up in summer and autumn (Pl. III, fig. 1). If no favorable water is in reach, the creature often ventures on a long migration to seek a spawning ground. This migration I have observed in some contiguous years, raking out the breeding pools which are two miles or even more apart, and found every year a large number of adults and spawns in the same pools, a fact which proves, how actively the migration is carried on by this slowly moving animal. The vast distribution of the species over the islands of Hokkaido, from the plains to the summits of high mountains is, therefore, due to nothing else than this tenacious character of the animal.

### **Pairing and Spawning Habits**

The pairing and spawning take place in water at the same time, and are connected with peculiar habits, as yet known in no other Urodelans. It is almost impossible to fully observe these habits in a natural pool inhabited by the salamander. The following observations have been made in aquaria under my control. At spawning, the gravid female seeks with zeal, a hard object, such as wood or gravel, or some other foreign bodies submerged in water. The female crawls at first very slowly about the object as if to seek a spot on it appropriate for its purpose. The next step in its behaviour is, that the cloacal opening is applied tightly to the spot which it has selected. Meanwhile the body is arched and the limbs are stretched sideway, and then an effort is made to glue to the spot the extremities of the egg-sacs which have just appeared from the vent. The final labour of the female is to draw out the whole of the sacs from the inside of its body, which it achieves by a backward movement with utmost exertion.

The attitude of the males present at this moment is of great interest. All the males in the vicinity, which till then have been quite indifferent to the female, now turn their heads toward the spawning female which they immediately approach. Arriving near the female they dart not upon the female itself, but upon the outcoming egg clusters. This rush is with an energy seen at no other time.

As to the subsequent behaviour of the males, I have noticed two different cases: in the first case the body is applied lengthwise to the egg-sacs which the female is discharging, while in the other case, the males coil or wind their body spirally around the sacs. In the former case they hang with the fore limbs to the fixed end of the egg sacs, and push the female backward with the hind limbs, working with the body bent and stretched alternately. With this action the males rub the egg-sacs with the lips of the vent, and assist the action, by which the sacs are drawn out of the female's body (Pl. II, fig. 2a). In the second case referred to, the sacs are scratched and kneaded by the males which are winding themselves round the sacs, and are further rubbed by the lips of the vent on the whole surface (Pl. II, figs. 1c, 2b-d).

Both the cases are to be assumed as nothing less than two important procedures, i. e. the procedure for thorough impregnation of the eggs in the sacs, and of assisting the delivery of the latter which is, therefore, the service of a midwife. These habits are by no means accidental, as one might suppose, but have been proved and affirmed by repeated observations and experiments. The most remarkable thing is, that the spawning takes place without assistance of males, but in this case it falls into a difficult delivery, and lasts several times as long as the ordinary case. Not only this, but the spawning may often end unsuccessfully, being caused by extraordinary swelling of the egg sacs due to their imbibition of water from outside before the liberation.

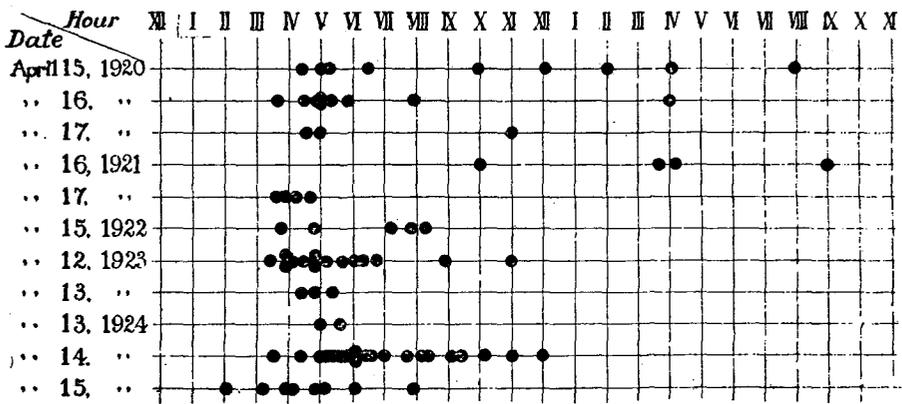
It seems almost improbable that the impregnation could be effected through such a thick layer of the substance of the egg sacs, but a series of direct observations show that the eggs discharged in the absence of any male never develop. This fact affords us the positive evidence of the external fertilization which occurs in the present species. External fertilization is known to be very rare in Urodelans in general, the only identical case which is proved, is confined to *Hynobius nebulosus* (Kunitomo 1910) and *Cryptobranchus alleghaniensis* (Smith B.G. 1912), whilst internal fertilization is very common as mentioned in *Plethodon cinereus* (Piesol 1909), *Salamandra atra* (Schwalbe 1896); *S. maculosa* (Gadow 1909), axolotl (Gasco 1881), *Amblystoma punctatum* (Smith B.G. 1907, Andrews 1897), and in many species of newts (Robin 1874, Gasco 1881, Bedriaga 1882, 1895, Zeller 1890, Gage 1891, Jordan 1893, Ritter 1897).

As soon as the spawning is over, the spent female seeks concealment under some objects as if for the purpose of resting, while the males hang several minutes more on the discharged eggs, subserving the impregnation,

and thereafter leisurely spend two or three hours around them. A scope of males around fresh spawns as occurs in the morning, shows that the same habit occurs also in the natural condition. I could obtain no evidence to confirm the view that the last habit of the males may be compared with the nursing habit as seen in *Autodax*, *Desmognathus*, *Amphiuma*, *Plethodon*, and *Cryptobranchus*.

Neither a sexual courtship nor any amplexus appears in the present species, although these habits are frequently reported in other Urodelans (Zeller 1891, Fischer-Sigwart 1896, Laesecke 1900, Hilton 1902).

The spawning takes place early in the morning, usually at day break. I have never seen breeding after 9 o'clock in natural pools. In captivity, however, it may occur even in the afternoon or at night, probably due to unfavorable condition of environment. In this respect the present species again differs from other Urodelans in which the spawning is said to occur at night (Hennicke 1895, Metzdorff 1896, Eycleshymer 1906, Wright 1908, Tago 1907, Gadow 1909). The following table shows the breeding hour in captivity:—



Text fig. 1. Breeding hour of *Hynobius lichenatus* observed in captivity. Each black spot along abscissae indicates the hour in question.

From this table, we know that the most frequent time of breeding is from about 4 am to 6 am, which agrees with the time under the natural condition as pointed out above.

### Spawns

The spawns are coated with double envelopes of jelly-like substance, both of which are products from the oviduct, corresponding to the secondary egg membranes in other Vertebrates. Of these envelopes, the inner, the egg-capsule, invests directly the chorion of respective eggs, while being itself enclosed together with other members within the other envelope, egg-sac. They nearly agree with the statements by Ikeda (1891) on *H. nigrescens*, by Tago (1907) on *H. naevius*; and by Kunitomo (1910) on *H. nebulosus*. According to Clark (1880) similar secondary egg membranes are also found in *Amblystoma*, while Shitkow reports the same in *Salamandrell*, and Tago (l. c.), in *Onychodactylus*; hence they constitute a characteristic common in all the genera of the Amblystomidae.

**Egg-sac.** At the time of the deposition it is an elongated, corrugated bag with a spirally twisted axis, measuring 55–60 mm. in length, and 10–13 mm in the greatest diameter. It is opaque and milky white in colour, due to thickly crowded microscopical granules found in the matrix. The superficial layer of the sac shows fine striations crossing one another at right angles. It is more or less elastic, and is much firmer in consistency than the underlying colloidal part which is in close contact with the egg-capsule in the interior. During the course of the development, the egg-sac swells owing to the colloidal part taking up water from outside; in accordance with this, the superficial corrugation smoothes down, till the whole sac assumes the shape of a sausage, attaining at last 230 mm. in length and 30 mm. in thickness. These changes in bulk are accompanied by further changes by which it becomes transparent and is loosened from the egg-capsules.

In sections the egg-sac almost agrees with the statement given by Kunitomo (l. c.) on *H. nebulosus*, except that in place of the dust-particles (*Staubpartikeln*) on the surface in this species I have found in *H. lichenatus* a green alga (*pleurococcus?*) living in its tissue as pointed out by Tago in *H. nigrescens* (l. c.).

**Egg-capsule.** It is spherical in shape throughout the development of the egg in contrast to the account by Kunitomo (l. c.) on *H. nebulosus*, according to which it afterward becomes oval in outline. In structure it resembles the egg-sac already mentioned, consisting of a firm and elastic superficial layer and a soft colloidal underlying substance which fits closely around the egg. As in other Amphibian spawns the colloidal substance takes up water very rapidly and in the course of one or two

hours a space appears on account of fluid filling between the capsule and the egg, so that the latter is now to orient itself with the animal pole uppermost. With the increasing fluid, the capsule grows larger and becomes more transparent. As a rule, in each capsule one egg lodges only, but sometimes two or even three eggs may be found.

**Chorion.** It is a very thin, transparent, structureless membrane, which is formed of the secretions from the ovarian follicles as described by Smith B. G. (l. c.) in *Cryptobranchius al'eghaniensis*. It is quite inconspicuous in uterine eggs due to a close contact with the egg, but becomes discernible when the egg is laid in water, being separated at the animal pole from the surface of the egg itself and bringing forth a fairly broad space which grows more spacious, as the development proceeds. A zona radiata could not be distinguished in a freshly laid egg as in *H. nebulosus* mentioned by Kunitomo (l. c.).

**Egg proper.** It is perfectly spherical, but when it rests quietly, the upper pole slightly flattens probably due to effects of gravity. The eggs of a given spawning are nearly uniform in size, but may vary in this respect according to different spawnings. The equatorial diameter of living eggs examined was 2.30–3.07 mm., the chorion inclusive.

The egg is chocolate brown in colour at the animal pole, grading into a gray or dull brown towards the vegetal pole. The intensity of the colour varies in eggs of different spawning, but is almost uniform in eggs from the same female.

Further, the eggs greatly vary in number according to different spawnings as well as between the two clusters of each spawning, and this with no definite rule. I have counted from 19 to 79 in each egg-sac.

### Embryonic and Larval Life

The development of an egg is exceedingly affected by the temperature, and oxygen amount, of surrounding water (Inukai, 1922). It lasts about three weeks in room temperature of 5°C–16°C, until the eggs hatch out. As to the ciliation and movement of a developing embryo within its chorion the species exhibits no marked difference from those hitherto described in other Amphibians.

The newly hatched larva which is about 13 mm. in length, has three pairs of external gills and a pair of balancers on the ventral surface of the head as in *H. nigrescens* (tago 1907). The larva spends its time in sunny parts of the water, without seeking any concealment, like the

adult. It is greenish brown above and lighter beneath in colour, with minute deep purplish brown dots of rather irregular distribution, which are crowded together into blotches on the body and tail, as the development proceeds (Pl. I., fig. 5).

### **Metamorphosis**

In the ordinary case the metamorphosis takes place during summer, but in higher altitudes or in colder waters is retarded till autumn or even the spring of the following year. The metamorphosis is expressed by atrophy of the external gills and median fin, by sudden development of limbs, by rapid protrusion of the eye-balls and by a change in pigment pattern, as usually occurs in other Urodelans. The atrophy of the gills goes on conjointly with the growth of the lung respiration which commences several weeks before metamorphosis is completed. As the lung-respiration is intensified the creature comes to the water surface more frequently than before. When the terrestrial life commences, the nonciliated stratified epithelium of the oral cavity gradually undergoes a change into a ciliated epithelium, continuous to that of the œsophagus, as is described by Gage (1891); the spiral larval gut is now greatly shortened, and the creature is less voracious than before.

The newly metamorphosed individual, 60-70 mm. in length, leaves the water and seeks shady and damp places like the full-grown adult; this characteristic continues to develop as the animal grows older. The limbs which are now very stout, are furnished with four fingers and five toes as is usual in the genus. But the fifth toe, coming forth later in the larval stage, is still rather rudimentary, representing the condition as mentioned in the type specimen. The ground colour of the body becomes rapidly deeper, and there appear distinct shining chromatophores in the dorsal and lateral surfaces. The purplish brown blotches increase in number especially on the head and trunk where they are confluent into lichen-like variegation. But new blotches being added in number one after another, soon later the variegation unites itself into a uniform coloration which is a characteristic of the adult, as mentioned previously.

### Neotenus Form

Among Amphibians the Axolotl is celebrated for its typical neoteny.\* This phenomenon is, of course, not confined to this form, but occurs very frequently among Urodelans as reported by many writers (Schreibers 1833, Thomas 1845, de Filippi 1861, Fatio 1864, Jullien 1868, Ebner 1877, Knauer 1878, Hamann 1880, Camerano 1883, Svetschkoff 1883, Kollmann 1885, Bedriaga 1891, Fischer-Sigwart 1896, Wolterstorff 1896, Zeller 1899, Stamm 1912, etc.). But hitherto in no other case than the Axolotl has such a thing been known that the reproduction takes place quite regularly in its neotenus form. Now *Hynobius lichenatus*, which is under notice, is by no means inferior in this respect to the Axolotl.

Until my affirmation of the typical neotenus reproduction in this species there is a long history to be reported. Some ten years ago, I collected a neotenus larva of this species floating dead on the water surface of the said mountain lake, *Kuttarush*; this was the first specimen which attracted my attention. A little later, I found three half digested specimens of the similar larva among the stomach contents of a salmonoid fish *Oncorhynchus nerka* which was also caught in the same lake. After this, similar specimens were repeatedly brought to me through the kindness of Mr. S. Nakao, Lecturer of our University, who lives near the lake. By these collections I convinced myself that the neotenus larvae might abound in the lake. To prove this I undertook dredging operations there in May of 1919, and my anticipation was realised by the haul which contained 25 living specimens of the larva in question. Some of those were easily recognizable as about to spawn by their gravid appearance. Besides these, the haul contained many fresh spawns which seemed at any rate to be those fertilized by some of the larvae collected; because they were found together with these spawns on one and the same weed which I pulled out of the lake. These researches created me the new belief that the larva certainly procreates as regularly as the Axolotl does in Lake Xochimilco in Mexico (Gadow 1908). This supposition was fully affirmed afterwards as described below.

The larvae from Lake Kuttarush were generally 150 mm. in length, whilst those from other localities measured hardly one third the length of

\*) The term "*Neoteny*" was first used by Kollmann (1882) to signify "the retention of larval characters beyond the normal period" particularly in Amphibians. But afterwards the meaning was extended by Camerano (1884) as well as by Boas (1896) to involve similar phenomena in all other organisms.

the former. Sometimes I found fairly large ones among over-wintered larvae from other waters, measuring about half the length of those from this lake, to which the neotenus reproduction was confined. The larvae from other localities have nothing to do therewith, as they always transformed into the adult form before the reproduction takes place, hence they may be excluded from our present thesis.

Of the neotenus specimens collected in the said lake, thirteen, 135–158 mm. in total length, were kept alive in aquaria under my control for about half a year from May 18th to November 13th, (Pl. I, fig. 3). At the time of the collection they were still very light in colour and had yet no shining chromatophore. The head, eyes, external gills and median fin could not be distinguished in shape from those of the ordinary larvae, the only criterion discriminative between these two forms being the size, and relative length of various parts.

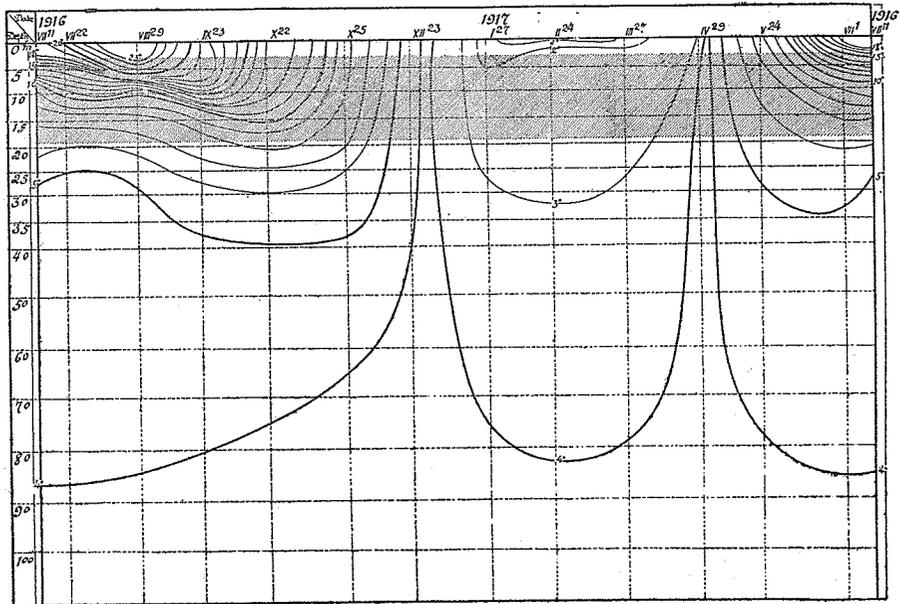
In the morning following the date of the collection, three of the thirteen neotenus larvae spawned in the aquaria in which they were kept, and the eggs developed into larvae; by these facts the neotenus reproduction in the present case became quite obvious. Similar collections and experiments were repeated year after year always with almost the same result as given above; these were, as might be easily implied, enough to prove the regular reproduction which takes place by the neotenus larvae in the lake. It is noticeable that the larvae from the neotenus breeding underwent metamorphosis in captivity of the room temperature as soon as they attained a certain length of body, and that they no longer repeated the neotenus reproduction by which they were brought forth. In this respect they are distinguished from the Axolotl which repeats very frequently the neoteny under a similar condition as above.

One more point to be noticed is what concerns the later fates of the thirteen neotenus individuals kept under my observation. Five of these performed a complete metamorphosis in the course of half a year; six showed some degrees of atrophy of the external gills and median fin (Pl. I, fig. 4), while the remaining two remained still in the larval condition until the winter came in. The gills were often nibbled off by each other, and lost parts were not repaired, differing from the results obtained by von Chauvin in experiments with the Axolotl (1876), according to which the neotenus adult does not metamorphose, and the lost parts of the gills are regenerated.

The larvae from neotenus parents agreed with the ordinary larvae in their undergoing metamorphosis at length, but differed from the latter in the duration of the larval life, which lasted about one month longer in the

former than in the latter under the same environmental conditions. The embryos and larvae from neotenus parents were as a whole active and healthy as those from the ordinary parents; the former were all alive during the period of the experiments, whereas many of the latter died.

Let us now turn a while to the physical as well as the biological conditions of Lake Kuttarush to explore any agencies suggestive of the occurrence of neoteny. The lake is a volcanic crater in origin, nearly circular in contour, surrounded by a range of mountains. According to Tanakadate and Kokubo (1918), it represents a water-basin with a narrow coastal shelf which is succeeded by a steep declivity into a flat bottom 120-146 meters in depth. The water level is about 279 meters above the sea. The water is very clear and pure, the transparency being 24.5 meters, the colour corresponds to No. 3 on Forel's scale. It is supplied by rain and by the springs of the surrounding mountains, no river feeds the lake and none empties from it. Due to, perhaps, this simple geological feature, the lake is under regular limnological changes, especially in respect to the temperature as shown in the following curve, which Mr. Tanakadate has kindly drawn for me.



Text fig. 2. Temperature condition of Lake Kuttarush in the years 1916 and 1917.  
The area with section lines corresponds to the thickly flourishing zone of *Nitella*.

The vegetation is limited to the coastal shelf, extending to a depth of 20 meters, beyond which no plant flourishes; the bottom consists of pumices, reddish sand and gravels of volcanic origin. The chief bottom flora of the coastal shelf consists of the following: *Nitella* sp., *Potamogeton maachianus*, *P. praelongus*, *Isoëtis echinospora* and *Myriophyllum spicatum*. Among these the first named, viz. *Nitella* sp. greatly predominates and grows some distance apart from the coast, its thickest flourishing part ranging from 3 to 19 meters in depth.

The fauna is also as simple as the flora. It consists of the salamander in question, *Oncorhynchus nerka*, insect larvae and plankton animals (*Anuraea cochlearis*, *Polyarthra platyptera*, ? *Triarthra longispina*, *Asplanchna periodonta*, *Scapholeberis* sp., *Cyclops strenuus*). The plankton is poor in amount as is proved by the transparency of the water referred to previously.

Such being the physical and biological condition of the lake, we will now concentrate our attention on the salamander under consideration. Long before the discovery of the neotenus form, I had been greatly interested with the lake in question, for the banks and the water of its immediate vicinity are inhabited so abundantly by the salamander that I could collect it by hundreds at a time. But these were always confined to the ordinary transformed form, and most of these specimens collected at the latter part of August were recently transformed individuals, signifying that they were certainly reared in this locality and had never undergone a long migration from other districts. This fact, though at the first glance of but small import, is yet in reality in connection with the present neoteny of most important significance. The banks, being steep slopes of the surrounding mountains, contain nowhere any quiet water favourable to the breeding of the salamander even at snow-break; this is also the case of the margins of the water, which are always disturbed by waves. Therefore, the above mentioned transformed form must have originated in a more central part of the lake, which is no other than the birth place of the neotenus form already alluded to. Further, direct evidence supporting this view is that nowhere else than in the before mentioned spot of the lake have the spawns of the salamander been found. So far as the transformed and neotenus form is of common origin in birth, we must admit a still further consideration that the nature of the banks have nothing to do with neoteny, at least of the present species. Therefore we must seek the cause of the present neoteny in conditions prevailing in other spots of the lake, laying aside the internal influence of the animal for the present.

At first I took the chemical nature of the water of the lake into the

consideration, but this was soon excluded from the present thesis by the results of the analysis which showed no marked difference in any part of the lake.\* That which next attracted my attention were the physical conditions of the habitat of the neotenus form and its spawning ground in the lake, which were, indeed, the only remaining environment connected with the neoteny to be sought for. Both the spots given above were easily known almost to coincide with the zone of *Nitella* mentioned before, through observation from a boat, dredging operation, and searching of the habitat of *Oncorhynchus nerka*, from the stomach contents of which I collected the neotenus specimens. Further, another proof supporting this view is that the stomach contents of the neotenus form consist chiefly of insect larvae which are found most abundantly in the *Nitella* zone.

In this last connection of *O. nerka*, special mentioning seems advisable. The fish was certainly never originally inhabited the lake but was introduced into it from a certain other lake some twenty years previously. Its first generation grew to a good size but the offsprings of these gradually diminished in size until they attained their present reduced length of 150-200 mm. Occasionally there have been caught, however, specially large individuals measuring over 350 mm. in length. These two forms of the fish show some difference in respect to their biological character: the smaller form is a plankton eater and is found everywhere in the lake sometimes even near the water surface, while the larger is the eater of the salamander in question, inhabiting, at least at night, in the *Nitella* zone. As far as I have examined the larger form contained only the neotenus salamander in the stomach, which suggests that its size is conditioned by its feeding upon the salamander, whereas the reduction in size in the other form is due to the poor plankton in the lake mentioned before.

Now, the problem remaining to be explored at present is the relation between the present neoteny and the physical conditions prevailing in the *Nitella* zone in the lake, which is indicated in Fig. 2 by section lines (p. 20). In order to solve this question I undertook a series of experiment; the results of which are given in the next chapter.

### Experiment

Larvae of the ordinary form, ranging 50-60 mm. in length, were divided into seven lots, each containing thirty individuals; *Limnodrilus* were sup-

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\* For the analysis of the water I am much indebted to Prof. Dr. E. Takahashi, to whom I hereby express my most cordial thanks.

plied as food, and the experiment lasted two months under the various conditions as follows. (1) The water temperature was retained at  $0^{\circ}\text{C}-4^{\circ}\text{C}$ ; and (2) likewise at  $4^{\circ}\text{C}-10^{\circ}\text{C}$ ; both lots were kept in a shaded spot. (3) The culture was made also in a shaded spot but with the water temperature  $11^{\circ}\text{C}-15^{\circ}\text{C}$ ; (4) the condition was made equal to the former, but the aquarium was put in a sunny spot, accordingly the water temperature rose much higher than in the preceeding ( $11^{\circ}\text{C}-19^{\circ}\text{C}$ ) each afternoon. (5) The culture was in a shaded spot, with a highly oxygenated water,\* of which the temperature was kept at  $4^{\circ}\text{C}-10^{\circ}\text{C}$ . (6) the water was likewise oxygenated and shaded, but with temperature of  $11^{\circ}\text{C}-15^{\circ}\text{C}$ . (7) Animals remained unfed throughout the period and kept in a sunny spot under the water temperature  $11^{\circ}\text{C}-19^{\circ}\text{C}$ . Lots I and II were in imitation of conditions prevailing in the deeper parts of Lake Kuttarush, whereas those of lots III and IV, to that of shallow pools found in the vicinity of Sapporo. The oxygenation experiment was attempted to make clear how oxygen, if appreciably emitted by vegetation, e. g. *Nitella*, reacts on the salamander larvae in connection with neoteny. The aquaria employed in the experiment were provided with perpendicular walls by which the escape of the captives was prevented. The results obtained are as follows:—

| Lot. | Applied conditions.  | Number of individuals metamorphosed. | Number of individuals half-metamorphosed. | Number of individuals not metamorphosed. |
|------|--|--------------------------------------|---|--|
| I    | Shaded, non-oxygenated; water temperature, $0^{\circ}-4^{\circ}\text{C}$ .           | 0                                    | 1   | 29                                       |
| II   | Shaded, non-oxygenated; water temperature, $4^{\circ}-10^{\circ}\text{C}$ .          | 2                                    | 1   | 27                                       |
| III  | Shaded, non-oxygenated; water temperature, $11^{\circ}\text{C}-15^{\circ}\text{C}$ . | 18                                   | 6   | 6  |
| IV   | Sunny, non-oxygenated; water temperature, $11^{\circ}\text{C}-19^{\circ}\text{C}$ .  | 29                                   | 0   | 1  |
| V    | Shaded, oxygenated; water temperature, $4^{\circ}\text{C}-10^{\circ}\text{C}$ .      | 21                                   | 2   | 7  |
| VI   | Shaded, oxygenated; water temperature, $11^{\circ}\text{C}-15^{\circ}\text{C}$ .     | 27                                   | 1   | 2  |
| VII  | Sunny, oxygenated; water temperature, $11^{\circ}\text{C}-19^{\circ}\text{C}$ .      | 0                                    | 0   | 30                                       |

\* Free oxygen was introduced into water in bubbles from a gas tank three times daily, i. e. 6 am., 2 pm., and 10 pm., each period lasting for one hour. The oxygen content of water of lot II was estimated at intervals after the introduction of the gas. It was 27.7065 cc. per liter of water just after the introduction, which decreased to 23.1936 cc. after one hour, and further decreased to 6.3612 cc. just previous to the next introduction. The last amount of oxygen was, however, far greater than that in the water of lot III, in which it was 2-3 cc. per liter of water. For these estimations I am much indebted to Mr. S. Kokubo, Assistant professor of the Tohoku Imperial University of Sendai, to whom my hearty thanks are due.

During the course of the experiment, an observation was made in respect to the rate of growth of the animals, which is appended below:— Lot I did not grow at all, whereas lot II increased in weight from 0.72 gm. to 1.56 gm. This difference was due to the different appetite of the animals: under such a low temperature as  $0^{\circ}\text{C}$ – $4^{\circ}\text{C}$ , the animals took food very rarely, and hence no appreciable increase in weight was produced. The most vigorous appetite was found in the animals of lot V, which was oxygenated under the water temperature  $11^{\circ}\text{C}$ – $15^{\circ}\text{C}$ ; accordingly they increased both in weight and length most rapidly among the lots cultured. The metamorphosis took place the earliest, therefore the average maximum size of the larval stage was not great, and was much below that of lot II. The latter, though kept under a low temperature, had fairly good appetite and attained the largest larval dimensions. The animals of lot VII which were unfed, decreased in weight from 0.78 gm. to 0.57 gm. and became quite emaciated. These animals also did not metamorphose, as shown in the above table.

Now, on examination of the results of this experiment we must notice two different agencies, i. e. 1) oxygenation, and 2) temperature, both of which profoundly act on metamorphosis as shown in the above table. Comparisons of lots II and III on the one hand, and of lots III and VI on the other, show us that the oxygenation accelerates metamorphosis, viz. the superfluous oxygen in water reacts on the salamander larva in a quite reverse way in connection with neoteny. This must be true in Lake Kuttarush. Even if any superfluous oxygen be emitted by vegetation in the lake bottom, the salamander larvae living therein shall be not brought to neoteny, but rather to a precocious metamorphosis. This proves that oxygen is by no means the agency for neoteny.

The next question which naturally arises in regard to this phenomenon, is the action of the other agency, i. e. temperature. A mere glance on the results in lots I, II and III is enough to accept how profoundly low temperatures act to retard metamorphosis. Sunny and shaded conditions (lots III, IV) speak also for this suggestion. This is still more evidenced by lots V and VI which both were oxygenated. An unique contradiction to this conclusion, is the results in lot VII, in which the metamorphosis is retarded even under high temperatures and sunny condition. But this retardation was effected by non-feeding: the animals remained small and did not grow up, hence requiring no consideration in our problem. An effect analogous to the last, is produced by exceedingly lowering the water temperature, as seen in lot I, in which case the appetite was greatly

diminished. Hence, low temperatures are to a certain extent a powerful agency for the retardation of metamorphosis, there should, however, be a definite degree in it, to bring about neoteny in the present case, a degree in which the appetite of the animals is still well preserved. In this respect, lot V was the best conditioned which is, after all, the only lot to be considered further on.

As given in the above table, the conditions applied to the last named lot, were shade, non-oxygenation, and water temperature at 4°C–10°C. Now, a spot of the lake fulfilling these conditions, is nowhere else than the *Nitella* zone mentioned before, particularly its deeper parts ranging 10–19 m. in depth, where such low temperatures as cited above, prevail all the year round. We are, therefore, justified in the conclusion, that neoteny must take place always in the similar physical conditions as just referred to, and that the primary factor of the conditions is ever-lasting low temperatures of certain degrees in connection with rich food supply.

Some additional notes are advisable in this connection: in the experiments metamorphosis was carried out in water, without regard to the nature of the walls of the vessel. This confirms the aforesaid view, that the nature of the banks of the lake has no connection with neoteny (see p. 21). The individuals invariably died, only when they were forced to remain a long time in water, the mortality increasing with the water temperature.

Further, individual variation is subject to the length of larval period under the same external conditions, and this occurs among the larvae from one and the same female, as seen by several lots given in the above table. The parallel case is reported by Wolterstorff (1896) in *Molge vulgaris* and *M. palmata*. This fact, as well as the peculiarities shown by the offsprings of the neotenus form (see p. 19), are data suggestive of the consideration, that neoteny may not be quite indifferent to inheritance of the animal.

### **Historical Review and Conclusion**

Since Duméril's discovery of the transformation of the Axolotl into *Amblystoma* (1867), various explanations have been attempted to make clear the causes by which neoteny is brought about. Weismann (1875) is of the opinion that the Axolotl in the Mexican lakes are prevented from becoming the perfect *Amblystoma* on account of the banks of these lakes, which are uninhabitable crusts of saline composition effected from extirpation of the surrounding forest. According to von Chauvin (1876) the Axolotl can comparatively easily be caused to develop further into the

perfect *Amblystoma* when put into such circumstances where it is enabled to breathe air more 'frequently' than under the ordinary conditions; a shallow vessel with insufficiently aerated water will lead to the same result. Hamann (1880) assumes from his collection of neotenus larvae of *Molge alpestris* from wells, that the retarded metamorphosis is brought about by lack of light and heat. Barfurth (1887) considers from his experiment, that a lower temperature retards the metamorphosis of Anuran tadpoles, while hunger accelerates the final stage of the larvae. Wolterstorff (1896) states that neoteny in *Molge alpestris*, *M. cristata* and *M. palmata* depends upon starvation, the effects of which differ according to different stages of the larvae. In *Molge marmorata*, metamorphosis is suppressed in the late-hatched larvae until the following year, on account of low temperature of the surrounding water as well as lack of food. Furthermore the larvae are adaptable to a wide range of external changes, which he refers to atavism as assumed by Weismann and Camerano (1883) in the Axolotl. According to Zeller (1899) neoteny in *Molge cristata*, *M. alpestris* and *M. taeniatus* is brought about by a too early winter, and the depth of the water inhabited by the animals, concluding this view from his collection of these salamanders from a deep pool with perpendicular walls and rain water 3 meters deep. Powers (1903), basing on the results of his experiment, claims that nutrition is the chief factor controlling the metamorphosis of *Amblystoma tigrinum*, all physical conditions having thereby but little to do with it. According to Gadow (1909) Shufeldt who observed the Axolotl under natural conditions in New Mexico, considers that such factors as rich supply of food, swamps drying up together with increasing temperature of the diminishing water, hurries metamorphosis, while deep water retards it. Gadow (1908) assumes from his observation in the famous Lake Xochimilco inhabited by the Axolotl, that the never-failing abundance of food and water, innumerable hiding-places in mud, under banks, and among reeds, all these features afford so great shelter, that the creatures may remain without disturbance and consequently retain all their larval characteristics which are not directly connected with propagation.

Besides the environmental conditions taken as the agencies bringing about neoteny, attention turned recently towards the internal causes leading the metamorphosis of Amphibians, and a series of investigations thereon were attempted as by Emerson (1905), Gudernatsch (1912, 1914, 1916), Uhlenhuth (1919, 1919a, 1920, 1920a, 1921, 1921a), Lenhart (1915), Allen (1916, 1917, 1918, 1920), Kaufman (1918), E. R. and M. M. Hoskins (1917, 1919), Swingle (1917, 1918, 1919, 1919a, 1919b, 1919c, 1920), Jenson (1916),

Graham (1916), Kendall (1918), Roger (1917, 1918), P. E. Smith (1917, 1916), etc. These studies on the whole prove, that the metamorphosis of Amphibians is greatly connected with the thyroid gland, thymus and hypophysis, as well as their interactions, although their influences are not quite equal upon all Urodelans and Anurans. According to investigations by Allen (1916, 1918) and Hoskins & Hoskins (l. c.) Anuran tadpoles which were thyroid-ectomized in an early embryonic stage, remained permanently in the larval condition, having in effects by the total inhibition of metamorphosis. According to Swingle (1919, 1919a, 1919b, 1919c), Anuran tadpoles fed with iodine or kept in its solution, accelerated metamorphosis in the same way as with the thyroid, and this effect of iodine was in relation with its quantity, i. e. the more the amount of iodine contained in food, the quicker metamorphosis occurs, whereas there occurs no metamorphosis in tadpoles fed with food destitute of this substance. From these facts, he owes too inhibition of metamorphosis in the Axolotl and other Urodelans, to the lack of iodine in the lakes inhabited by them. From his own experiment, in which iodine solution did not accelerate metamorphosis, Uhlenhuth (1921) explains the question, on the other hand, as follows: in contrast to the frogs in which, as just stated, the factor for metamorphosis is confined to the thyroid glands, the salamander larvae require in this regard one more factor, viz. a certain function releasing the hormone from their follicles and ever-prevailing low temperatures, by which metamorphosis is suppressed, lead the Axolotl to the neoteny, as in the case of the Rocky Mountains. This view is, however, an assumption which may require further demonstration.

As to the relation between metamorphosis and sexual maturity in Amphibians, several researches have been attempted. Swingle (1918), Allen (1918), and Hoskins & Hoskins (1919) agree with one another in that these two changes are little related to each other. Uhlenhuth (1919a, 1921) explains this maintenance as follows: the temperature coefficient with chemical function for the development of the sexual organs is lower than that for the elaboration of releasing factor of the thyroid hormone, viz. the sexual maturity is reached in low temperatures, having no connection with metamorphosis.

From the historical review of literature given above, we know that all the views hitherto afforded as regards the origin of neoteny, diverge from the results of my present research, except those of Barfurth! (1887) and Uhlenhuth (1922). These two writers affirm low temperatures to be the efficient agents in inhibiting metamorphosis without, however, taking into

consideration a certain grade of temperature, in which the appetite of the animals must be preserved in order to induce neoteny.

Powers' opinion before mentioned diverges from my results on the affection of the physical conditions upon metamorphosis which he denies positively owing probably to his inaccurate method of oxygenation and temperature regulation. Gadow's view (l. c.) agrees with mine as far as concerned to a rich food supply which is necessary to cause neoteny, and diverges in his neglecting to take temperature as a powerful controller of metamorphosis, consequently of neoteny. Von Chauvin assumes (l. c.) that insufficiently aerated water in shallow vessels brings about a precocious metamorphosis, while in reality the oxygenation accelerates transformations. As to walls or banks of waters, which are put in connection by Zeller and Weismann with neoteny, there is no fact to be assumed as such; on the contrary transforming individuals enforced to live in water, for a long time, died invariably. The criticism upon endocrinological conceptions hitherto advanced in respect to metamorphosis lies out of the scope of our present problem.

Now, in the results of the present investigation, the primary environmental factor which brings about neoteny, consists in a long-lasting certain low temperature which acts on the salamander larvae, connected with rich food supply in the habitat, namely bringing forth an extraordinary large larval form associated with sexual maturity which is independent of metamorphosis. This well explains the difference between ordinary pools and Lake Kuttarush regarding the mode of reproduction of the *Hynobius* in question: in the former waters, due to their minor depth, the water temperature though very low at the spawning period of the salamander, becomes much higher with the progress of the spring, finally reaching the condition which brings about the metamorphosis of the larvae. In Lake Kuttarush as mentioned before, the spawning ground is limited to the vegetation zone, in which the salamanders develop and grow with rich supply of food. The larvae which inhabit in the deeper parts of the *Nitella* zone, grow into the neotenus form, being constantly exposed to low temperatures of proper degrees, whereas those living in the shallow parts of the zone go to metamorphose, owing to rising water temperature in summer, nearly agreeing with the conditions as found in ordinary pools. The metamorphosing larvae seek the margin of the lake as a general behavior of salamanders in general, and land on the banks where I collected numerous specimens of the adult form.

Lastly, I express my most cordial thanks to Dr. Hatta, for his kind suggestions given in the course of the present investigation. In fact, he

was the first to collect and observe the spawn and adult of the species under consideration and to turn attention to this problem. My obligations are also due to Mr. T. Hikita, an ichthyologist of our University, by whose help I have been able to make so large a collection of the adult form as already enumerated.

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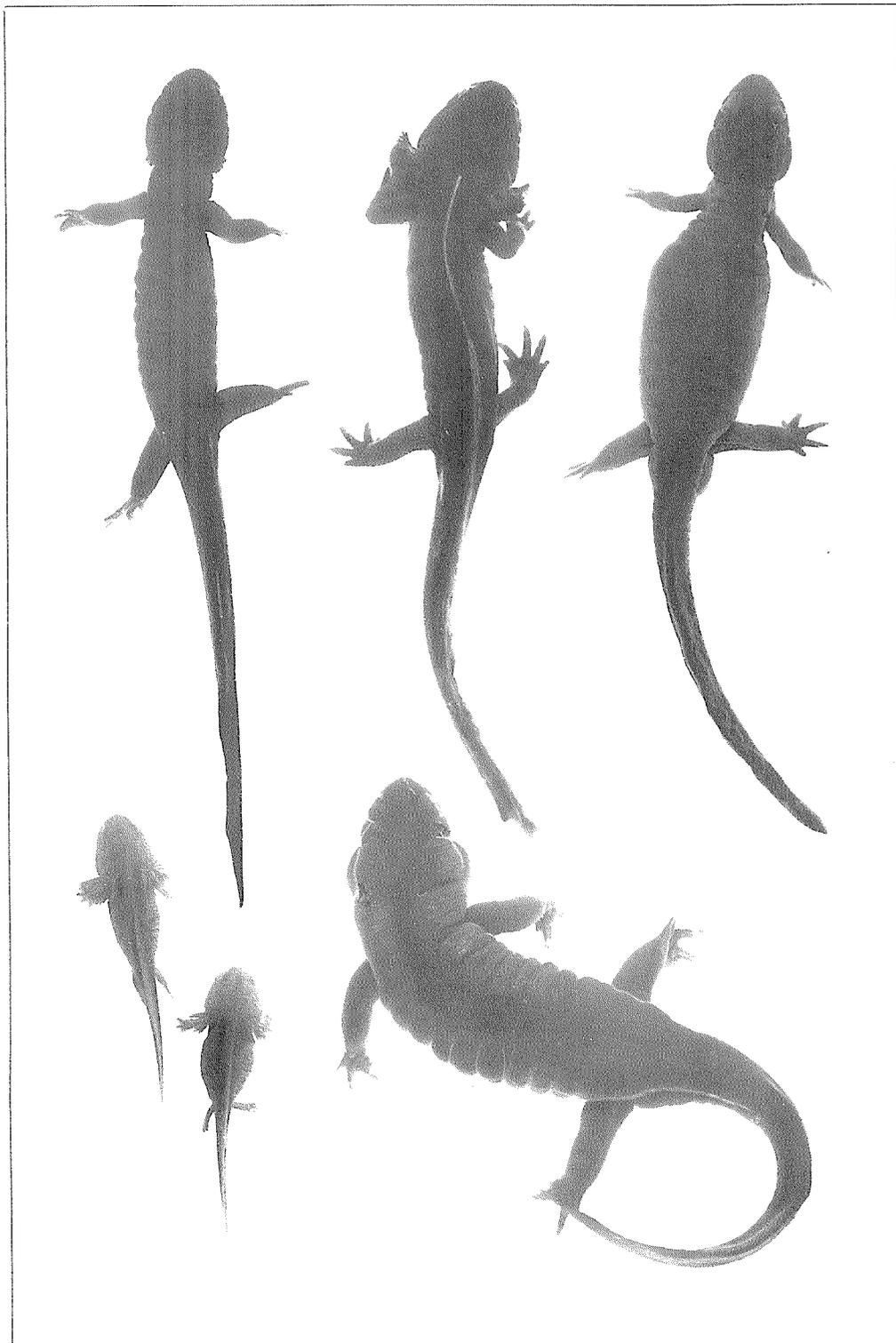
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**Plate I.**

- Fig. 1. Fully mature male; slightly reduced.
- Fig. 2. Gravid female; slightly reduced.
- Fig. 3. Neotenuous female; slightly reduced.
- Fig. 4. Metamorphosing individual of the neotenuous form, with stumps  
of the external gills; slightly reduced.
- Fig. 5. Ordinary larvae; nat. size.



*Sasaki photo.*

## Plate II.

- Fig. 1a. Female (2) delivering her spawns (3), to which two males (4) are turning their head. 1. Previously deposited spawns; 6. pregnant female (appearing also in Figs. 1b & c).
- Fig. 1b. Spent female (2) away from her spawns. Two males (5) which correspond to 4 in Fig. 1a, now roping themselves with the newly deposited spawns (3).
- Fig. 1c. Males (5) continuing their impregnation on the spawns (3).
- Fig. 2a. Female (8) delivering her spawns (11). Midwifing male (7) applying his body to the outcoming egg sacs. Another male (9) approaching the egg sacs.
- Fig. 2b. Spent female (8) seeking concealment under a stone. Three males (10) roping themselves with the egg sacs (11).
- Fig. 2c & d. Males (10) continuing their impregnation on the egg sacs (11).

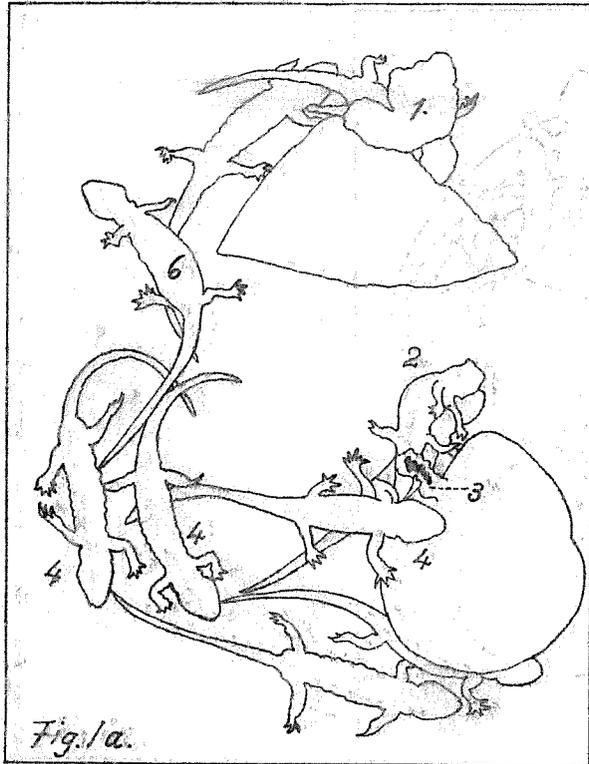


Fig. 1a.

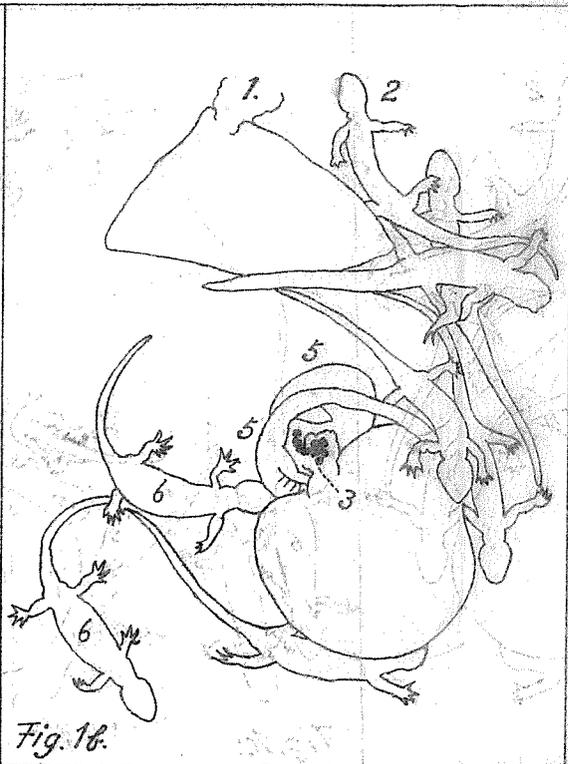


Fig. 1b.

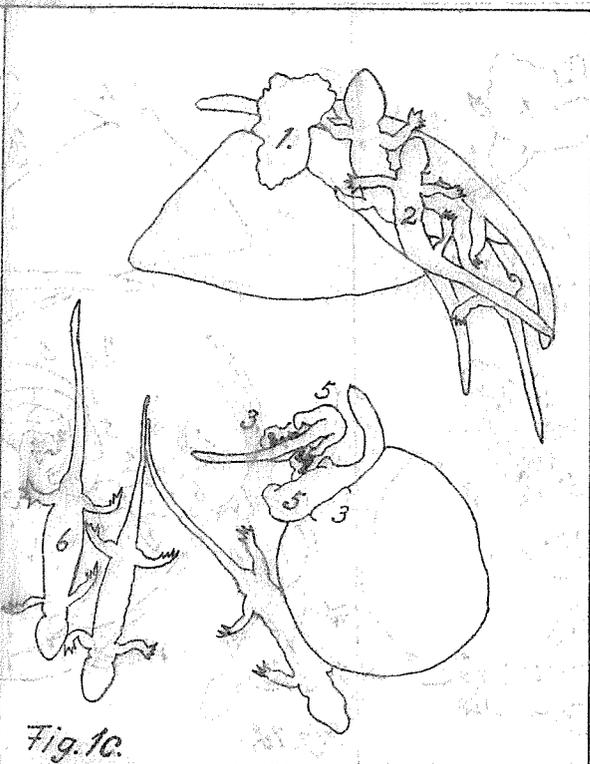


Fig. 1c.

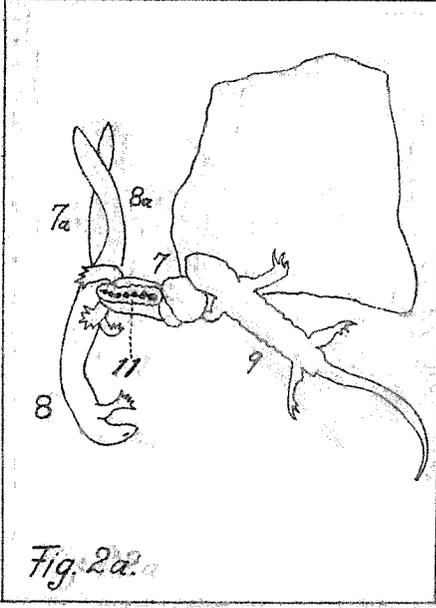


Fig. 2a.

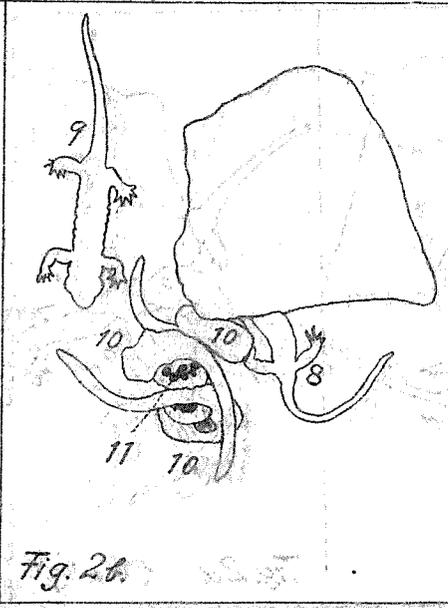


Fig. 2b.

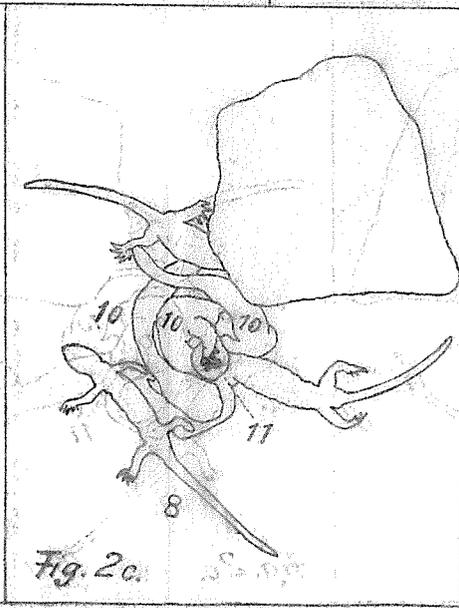


Fig. 2c.

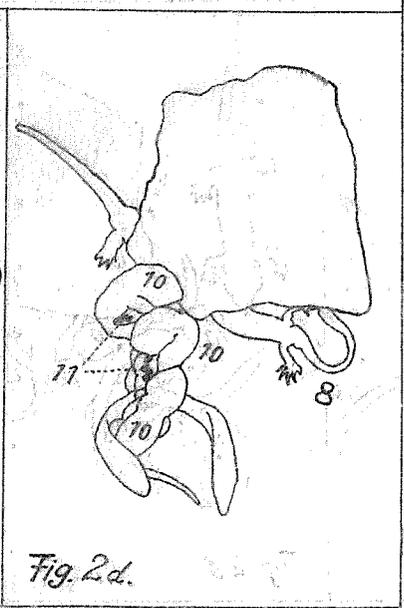
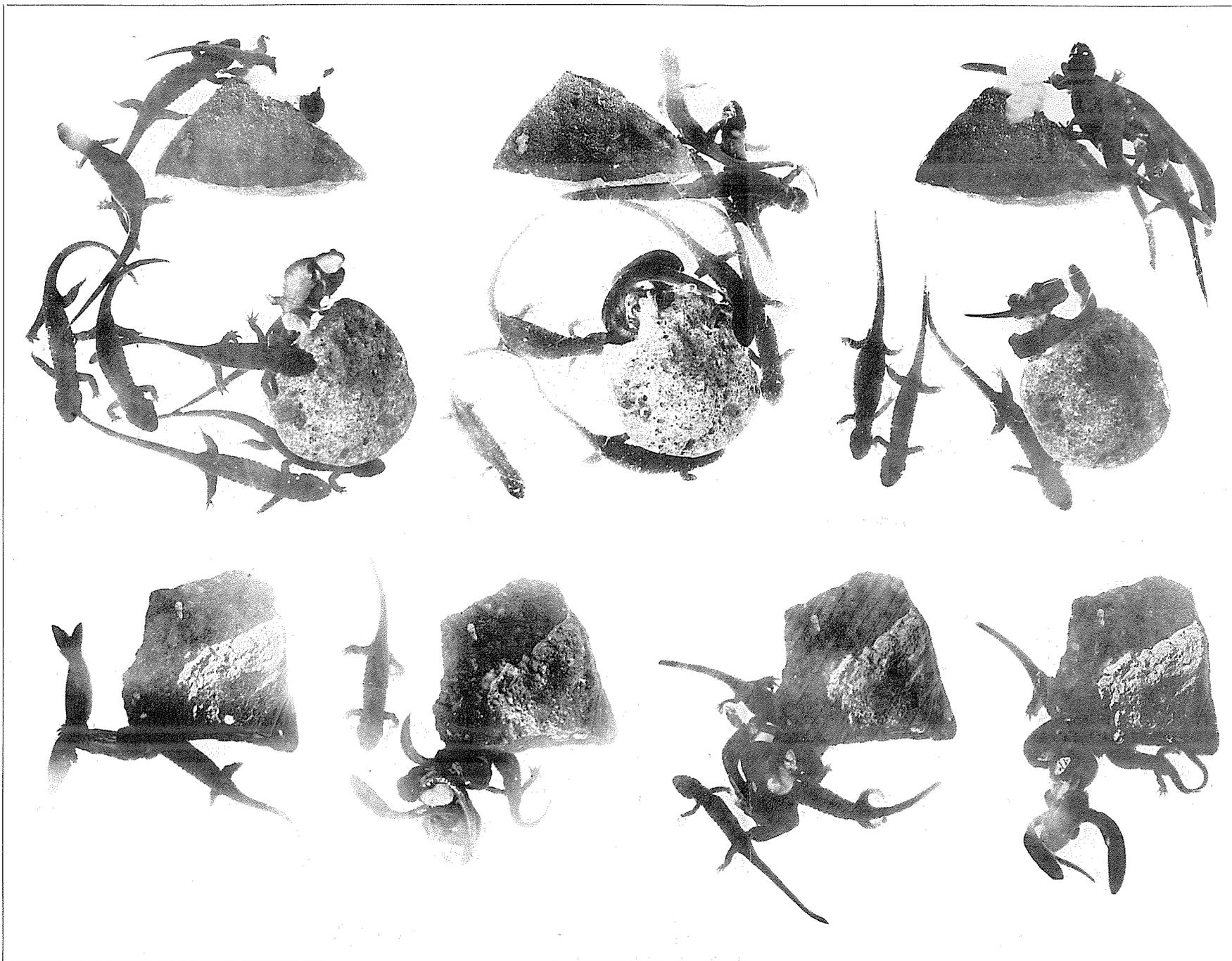


Fig. 2d.



Sasaki photo.

**Plate III.**

- Fig. 1. Transient pool near the foot of Mt. Moiwa, still with floating ice on the surface. The spawns of *Hynobius lichenatus* are fastened to shrubs hanging over the pool. At the time of their deposition the shrubs were submerged but now appear due to decreasing water of the pool.
- Fig. 2. Neotenous individuals, with their spawns glued to *Nitella*.



Sasaki photo.