Changes in the Size of Egg-form Embryos with the Advance of Development in the Salamander *Hynobius nigrescens*

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Abstract: Using the eggs derived from 16 females of *Hynobius nigrescens*, changes in the size of developing egg-form embryos (from 2-cell embryo to gastrula) were studied. No significant difference in the proportional increase in size was found among the embryos of any of the females examined. Conversion factors for obtaining the initial egg size from the size of egg-form embryos were presented.

Key words: Caudata; *Hynobius nigrescens*; *Hynobius lichenatus*; egg-form embryo; egg size; ontogenic increase

Amphibian embryos that maintain an egg-like appearance during the earlier periods of development are subject to size changes (Løvtrup, 1960). Collecting uncleaved urodelan eggs in the field is rather difficult. It is therefore necessary to make a correction in measured values when the uncleaved egg size is estimated from the size of the egg-form embryos (2-cell embryo to gastrula). Kaplan (1979) presented the conversion factors for each developmental stage for obtaining the initial egg size in *Ambystoma tigrinum* and *A. maculatum*. In the present study, the ontogenic increase in the size of egg-form embryos was examined in *Hynobius nigrescens*, as well as in *H. lichenatus* for comparison.

MATERIALS AND METHODS

Eggs derived from 16 females of six populations of *H. nigrescens*, which were collected in breeding ponds at various altitudes in the Niigata area, were used as materials. For comparison, eggs derived from two females from a single population of *H. lichenatus* were used. In every clutch, 15 fertilized eggs were artificially inseminated and drawn from the egg capsule, were placed individually in 10% Holtfreter's solution, and kept at 16°C. The diameter of the egg-form embryos was measured, along two lines crossing at right angles, in each stage. For the determination of embryonic stages, the normal table for de-

velopment in *H. nigrescens* (Usui and Hamasaki, 1939) was used. The measurement of embryos was done to the nearest 0.01 mm under a binocular microscope, and the average in each clutch was calculated for every developmental stage.

RESULTS AND DISCUSSION

Table 1 shows the mean diameter of uncleaved eggs derived from each female and the characteristics of sampling sites. The range of the mean egg size was rather wide (from 2.37 to 3.06 mm). However, the proportional increase in the size from the developmental stages up to gastrula did not significantly differ among the embryos of all the females examined (Friedman two-way analysis of variance). Fig. 1 shows the increases in the size of egg-form embryos with the advance of development in several females of *H. nigrescens* and *H. lichenatus*. In this figure, it can be seen that the trajectory forms of increasing embryo size were similar in *H. nigrescens* in spite of considerable variations in

FIG. 1. Means (circles) and standard deviations (dashes) of the diameters of eggs and embryos derived from the females collected in Yahiko-jinja-ike, Rintsuji-ike and Tagami-sawa. Solid circles: *H. nigrescens*, Open circles: *H. lichenatus*.

TABLE 1. Sampling sites and averages of initial egg diameter for each female.

Sampling sites	Locality	Altitude (m)	Date of collection	Initial egg size (mm)		
<i>Hynobius nigrescens</i>						
Rintsuji-ike	Yahiko-Mura	40	Mar. 10	2.82	2.47	2.56
Yahiko-jinja-ike	Yahiko-Mura	60	Jan. 21	2.53	2.55	—
Shichiko-ike	Kakizaki-Machi	520	Apr. 21	2.67	2.67	—
Gaba-ido	Shitada-Mura	630	May 7	3.02	2.99	2.50
Nihondaira-san-ike	Mikawa-Mura	650	May 14	2.97	2.96	2.98
Wakakusa-ike	Shiozawa-Machi	800	May 19	2.37	2.47	2.39
<i>H. lichenatus</i>						
Tagami-sawa	Tagami-Machi	80	May 24	2.82	3.06	—

the initial egg size. Between *H. nigrescens* and *H. lichenatus*, it seems, however, that the trajectory form of embryonic size differs somewhat, particularly in earlier developmental stages. Although no statistical difference is found between *H. nigrescens* and *H. lichenatus*, it is estimated that the proportional increases in

size between stages differ somewhat between these two species. In addition, the trajectory form of increasing embryonic size in *H. nigrescens* differs slightly from the results reported in *Ambystoma* (Kaplan, 1979).

The conversion factors for *H. nigrescens* by which embryonic size should be multiplied in order to obtain the initial egg size are presented in Table 2. It seems that these conversion factors are uniformly useful in *H. nigrescens*. It may be possible to use them practically for *H. lichenatus* also, except in the earlier developmental stages.

TABLE 2. Conversion factors for calculating initial egg diameter from the diameter of egg-form embryos

Normal stage*	Description of stage	Conversion factors
1	Uncleaved	1.00
2	2-cell	0.98
3	4-cell	0.97
4	8-cell	0.96
5	16-cell	0.96
6	32-cell	0.95
7	Morula	0.95
7+	Late morula	0.94
8	Early blastula	0.94
8+	Early blastula	0.94
9	Mid blastula	0.93
9+	Mid blastula	0.93
10	Late blastula	0.92
10+	Appearance of blastoporic lip	0.91
11	Crescent blastoporic lip	0.90
12	Yolk plug	0.90

* Usui and Hamasaki (1939)

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要旨 クロサンショウウオ *Hynobius nigrescens* の球状胚の発生にともなう大きさの変化

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両生類の卵は発生の進行と共に大きさを増す。よって、卵サイズを正確に知るためには受精直後の未分割卵を用いることが求められるが、野外で受精直後の卵を採集することは必ずしも容易でない。そのため比較的に採集の容易な球状胚の大きさから産出直後の未分割卵の大きさを算出する方法を検討した。標高 40~800 m の産卵池から採集したクロサンショウウオの雌16個体に由来する卵、および比較のためにトウホクサンショウウオの雌2個体に由来する卵を用いて、囊胚期までの胚の発生に伴う直径の変化

を調べた。受精卵の大きさは直径 2.37 mm から 3.06 mm までと大きく異なったが、ステージ間の直径の増加の割合は、全ての雌由来の卵の間で有意な違いはみられなかった。しかし、クロサンショウウオとトウホクサンショウウオの間には、ごく初期のステージにおいて、サイズの増加の割合に多少の違いがみられた。各ステージの胚の直径から未分割卵の直径を算出するための係数を示した。

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