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Short Report

Effect of Temperature on Embryonic and Post Embryonic Development of Salamander, *Hynobius retardatus*

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Abstract In order to elucidate the environmental factors that caused the natural neoteny of *Hynobius retardatus*, the effect of temperature on the embryogenesis, the larval growth and the metamorphosis of this amphibian was studied. The embryogenesis was completed even at 4°C, although the embryonic development was considerably delayed. At 10°C, the larvae grew very slowly, and its metamorphosis never occurred until 200 days, resulting in an extraordinarily large larvae in size. At 4°C, the growth of the larva was considerably delayed and no larvae could be metamorphosed even in the cultivation for more than one year. These observations suggest that 10°C is a critical temperature for metamorphosis.

Introduction

A salamander, *Hynobius retardatus*, is the Urodele Amphibian widely distributed in Hokkaido Island. In 1924, Sasaki first collected several *Hynobius retardatus* at Lake Kuttara (1), which exhibited features characteristic of "neoteny" (2). Since the water temperature at this lake is low (5°~10°C) and almost constant throughout a year (3), it has been assumed that the neoteny-like salamanders result from such a low temperature environment (1). In general, the metamorphosis of this animal takes place during summer. It is known, however, that in high altitude or in cold water, the metamorphosis is retarded, which consequently occurs in autumn or sometimes in the next spring.

In this preliminary study, we examined the effect of various temperatures on embryogenesis, the growth and metamorphosis of *H. retardatus*.

Materials and Methods

*Hynobius retardatus* and *Rana chensinensis* eggs were collected from the ponds near Sapporo. They were divided into three groups from one litter and were raised at 4°C, 10°C and 22°C, respectively, in tap water.

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2 The Institute of Low Temperature Science, Hokkaido University, Sapporo, 060
After hatching, *Rana chensinensis* tadpoles were fed on blodd spinach, and *Hynobius retardatus* larvae were fed on *Tubifex hattai*. The body length was measured every ten days.

**Results and Discussion**

Statistical test (Kruskall-Wallis test) displayed the significant difference in size among newly hatched larvae (at 43rd stage according to Usui and Hamasaki (4)) at various temperature, which were raised at 4°C, 10°C and 22°C (Table 1). The embryonic period depended on temperature; the higher temperature the shorter embryonic period. However, the largest larvae were obtained from cultivation at 10°C, and the size of the larvae at 4°C was significantly larger than that of larvae at 22°C. The above observation suggests that the metabolic efficiency may be varied with temperature, since the total amount of energy reservoir and the compounds including proteins contained in the eggs should be equal at the initial stage, and the environment at 10°C seems optimal in terms of embryogenesis of this animal.

**Table 1.** Time to hatching at various temperature and body length at the hatching

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time to hatching</th>
<th>Body length at hatching (43 stage) (mm)</th>
<th>Rana chensinensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>66~80 days</td>
<td>18.6±0.9 (n=21)</td>
<td>⋯⋯</td>
</tr>
<tr>
<td>10°C</td>
<td>30 days</td>
<td>22.3±0.7 (n=18)</td>
<td>14 days</td>
</tr>
<tr>
<td>22°C</td>
<td>10 days</td>
<td>17.0±0.5 (n=18)</td>
<td>4 days</td>
</tr>
</tbody>
</table>

⋯⋯ could not “hatch out”
mean±S.D. n=number

*Rana chensinensis* developed earlier at 10°C and 22°C than *H. retardatus* did. However, this frog could not hatch out at 4°C. These results may reflect the native spawning of the two species; *R. chensinensis* spawn usually in sunny shallow pools, while *H. retardatus* spawn in cold water with floated ice or snow.

Figure 1 shows the effect of temperature on the growth and metamorphosis of *H. retardatus* larvae after hatching. At 22°C, the larvae grew most rapidly and the metamorphosis occurred, at the size of 55 mm, about 50 days after hatching. Contrary, the larvae which were kept at 10°C were not metamorphosed even after 200 days, and continued to grow beyond the size of 22°C larvae at metamorphosis. At 4°C, the larvae grew most slowly and the metamorphosis never occurred even after more than one year, and will not be metamorphosed forever.

The cultivation of larvae at 4°C for almost one year after transfer from the initial cultivation at 10°C for 240 days allowed us to obtain an extraor-
ordinarily big larvae 90 mm in body length (usual size is about 55 mm at metamorphosis). Sasaki reported that neotenous form of this species is as big as 150 mm (1). We are interested of what will happen with the extraordinarily big larvae obtained in our laboratory, particularly in their reproductive system, if they are further cultivated at 4°C.

Acknowledgments

I wish to thank Prof. H. Chino for reading through the manuscript.

Literature Cited