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Short Report

Effect of Temperature on Embryonic and Post Embryonic Development of Salamander, *Hynobius retardatus*[1]

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Abstract In order to elucidate the enviromental facters that caused the natural neoteny of *Hynobius retardatus*, the effect of temperature on the embryogenesis, the larval growth and the metamorphosis of this amphibia 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 occured until 200 days, resulting in an extraordinally large larvae in size. At 4°C, the growth of the larva was considerably delayed and no larvae could be metamorphosed even in the caltivation 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 enviroment (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 occurrs 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 devided 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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After hatching, *Rana chensinensis* tadpoles were fed on boild 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 caltivation 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 enviroment 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

	Hynobius retardatus		*Rana chensinensis*
Temperature	Time to hatching	Body length at hatching (43 stage) (mm)	Time to hatching
4°C	66~80 days	18.6±0.9 (n=21)	……
10°C	30 days	22.3±0.7 (n=18)	14 days
22°C	10 days	17.0±0.5 (n=18)	4 days

…… 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 alomst one year after transfer from the initial cultivation at 10°C for 240 days allowed us to obtain an extraor-

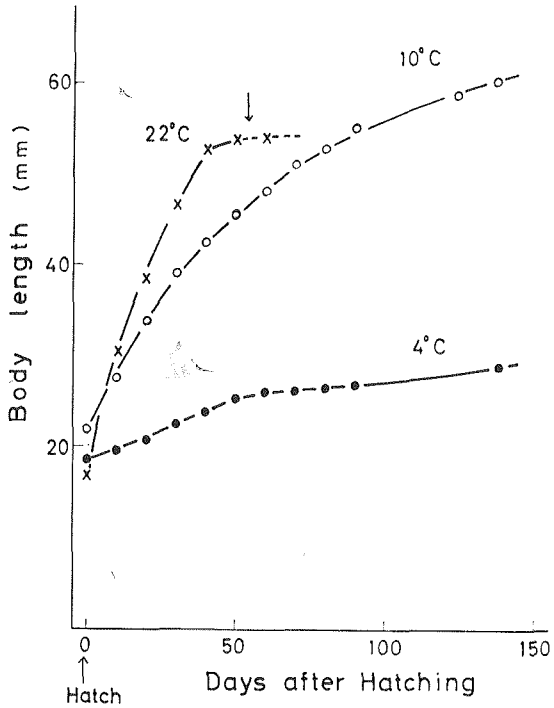

Fig. 1. Effect of temperature on the growth and metamorphosis of *Hynobius retardatus*. Arrow shows metamorphosis.

dinally 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 extraordinally big larvae obtained in our laboratory, particularly in their reproductive system, if they are further cultivated at 4°C.

Acknowledgments

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