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# Larval Life History Variation in Two Populations of the Japanese Salamander *Hynobius kimurae* (Amphibia, Urodela)

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**ABSTRACT**—Larval life histories of Kyoto and Tokyo populations of the stream breeding salamander *Hynobius kimurae* are compared in the field. The Kyoto population has smaller adults and ovarian egg numbers, but larger egg sizes. Minimum water temperature is lower, and the stream partly dries up between late October and late November in Kyoto, while the amount of water is always stable in Tokyo. In Kyoto, metamorphosis occurs between late July and late September and no larva is found in the water in and after October. In Tokyo, only 21% of larvae metamorphose in the first year and most overwinter and metamorphose between late May and mid June of the second year. Size of hatched larvae does not differ between Kyoto and Tokyo, but metamorphs from Kyoto are smaller than first year metamorphs from Tokyo, which in turn are smaller than the second year metamorphs. Differences seen in larval life histories between the two populations may represent adaptations for differential stability of water level and water temperature, and resultant differences in the size of metamorphs possibly induce differential adult body size, and hence fecundity.

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## INTRODUCTION

Variation in amphibian larval traits such as growth rates, size at metamorphosis, and the timing of metamorphosis influence postmetamorphic mortality (Smith, 1987), age and size at first reproduction (Berven and Gill, 1983; Semlitsch *et al.*, 1988; Tilley, 1980), and reproductive strategy (Howard, 1978). Environmental factors such as water temperature and food level can strongly influence larval growth and development rates (e.g., Smith-Gill and Berven, 1979; Berven and Gill, 1983); however, a genetic component may also contribute to interpopulational variations in larval growth and development (e.g., Berven, 1982; Juterbock, 1990; Austin and Camp, 1992).

Among salamanders, most studies of larval life histories have been conducted on American plethodontids and ambystomatids (Harris *et al.*, 1990; Hutcherson *et al.*, 1989; Nussbaum and Tait, 1977; Semlitsch *et al.*, 1990; Sexton and Bizer, 1978; Tilley and Bernardo, 1993), but very few studies have been conducted on Asian hynobiids which include evolutionarily primitive members (e.g., Kusano, 1981; Hayase and Yamane, 1982; Takahashi and Iwasawa, 1990).

*Hynobius kimurae* from Honshu, the main island of Japan, spawns from February to April. Some of the hatched larvae metamorphose before late September, but others overwinter and metamorphose the following year (Matsui, 1981; Sato,

1943). Adult body size of *H. kimurae* varies geographically (Matsui, 1981), and populations from Kyoto and Tokyo differ markedly in this respect. It is uncertain whether larval life histories including growth, development, and timing of metamorphosis also vary between these populations. In this report, we compare larval life histories of these two populations from field data, and discuss factors that may contribute to interpopulation variation in larval and postmetamorphic life histories.

## MATERIALS AND METHODS

Field surveys were made between 1992 and 1994 in small montane streams of Kurama-Ninose-cho, Sakyo-ku, Kyoto (35°08'N, 135°45'E, 550m elev.) and Miyama-cho, Hachioji-shi, Tokyo (35°42'N, 139°13'E, 450m elev.) where *H. kimurae* breeds. In order to document the spawning season and larval growth and development, we made surveys every 14 days in Kyoto and every 20 days in Tokyo from March 1992 to May 1993. To gather data on embryonic development, we set a plastic case (25 × 20 × 20 cm) at the bottom of a pool at each site and put several egg sacs in them. We periodically collected and examined embryos in cages and measured water temperature of the stream 5 cm under the surface of these pools.

We collected larvae at the sites until all larvae had metamorphosed and none was found in the stream. About 15 larvae were randomly collected for each sampling, fixed in 10% formalin and later stored in 5% formalin. Developmental stage was determined following tables for the congeners *H. nigrescens* (Iwasawa and Yamashita, 1991) and *H. lichenatus* (Sawano, 1947). Measurements of snout-vent length (SVL) were made from the tip of the snout to the anterior corner of the cloaca using a digital caliper to the nearest 0.05 mm.

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Table 1. Comparisons of snout-vent length (SVL in mm), ovarian egg number (OEN) and egg diameter (ED in mm) in two populations of *Hynobius kimurae*

Locality	Male SVL			Female SVL			OEN			ED			
	n	mean	2SE	n	mean	2SE	n	mean	2SE	n	mean	2SE	
Kyoto	20	63.0	1.55	15	72.7	2.02	15	26.9	2.41	15	4.5	0.10	
Tokyo	20	71.2	1.23	15	76.8	1.86	15	32.4	4.22	15	4.3	0.08	
<i>U</i> -test	( <i>P</i> )	< 0.01			< 0.01			< 0.05			< 0.01		

In order to determine relationships between adult body size and reproductive parameters, adults congregating in breeding streams were collected during December and April from 1992 to 1994. In both Kyoto and Tokyo, 20 males and 15 females were collected. Measurements for adult SVL were made as for larvae. Ovarian eggs were removed and fixed in 10% formalin. Each of 10 eggs haphazardly chosen from a female was measured for maximum and minimum diameters and the average was used to estimate egg diameter. The average diameter for all 10 eggs was used as the egg diameter for each female. For statistic analyses, Mann-Whitney *U* tests were utilized. In order to examine relationships between parameters, analysis-of-covariance (ANCOVA) was performed. The significance level was set at 0.05.

## RESULTS

### Adult body size, ovarian egg number, and egg diameter

Adult *H. kimurae* aggregated near the spawning site before the snow season and remained there during the winter. Mean SVLs of both sexes of adults from Kyoto were significantly smaller than those from Tokyo (Table 1). Ovarian egg number was significantly smaller and egg size significantly larger in females from Kyoto than those from Tokyo. In both Kyoto and Tokyo populations, a positive correlation was found between female SVL ( $X$  mm) and egg diameter ( $Y$  mm; Kyoto:  $Y = 0.032X + 2.174$ ,  $r = 0.66$ ,  $n = 15$ ,  $P < 0.01$ ; Tokyo:  $Y = 0.029X + 2.077$ ,  $r = 0.72$ ,  $n = 15$ ,  $P < 0.01$ ). In contrast, no correlation was detected between female SVL and ovarian egg number (Kyoto:  $r = 0.02$ ,  $P > 0.05$ ; Tokyo:  $r = 0.02$ ,  $P > 0.05$ ), or between egg diameter and ovarian egg number in either population (Kyoto:  $r = 0.04$ ,  $P > 0.05$ ; Tokyo:  $r = 0.09$ ,  $P > 0.05$ ).

### Breeding habitat

In both Kyoto and Tokyo, *H. kimurae* laid eggs in the headwaters of small streams. The points of the streams where egg sacs were found were  $< 1$  m in width and 10-20 cm in depth in Kyoto and  $< 1.5$  m in width and 20-30 cm in depth in Tokyo. Seasonal water temperatures did not exceed over  $20^{\circ}\text{C}$  in either locality, while the minimum temperature was  $2^{\circ}\text{C}$  in Kyoto and  $5.5^{\circ}\text{C}$  in Tokyo (Fig. 1). The two localities differ in water temperatures, especially in winter; the average temperature between December of 1992 and March of 1993 was  $3.6^{\circ}\text{C}$  in Kyoto but  $6.6^{\circ}\text{C}$  in Tokyo. In Kyoto, water temperatures rose from  $2$ - $4^{\circ}\text{C}$  during late January and early March when small number of eggs were found, to  $6$ - $8^{\circ}\text{C}$  during mid March and early April when egg laying was at its peak.

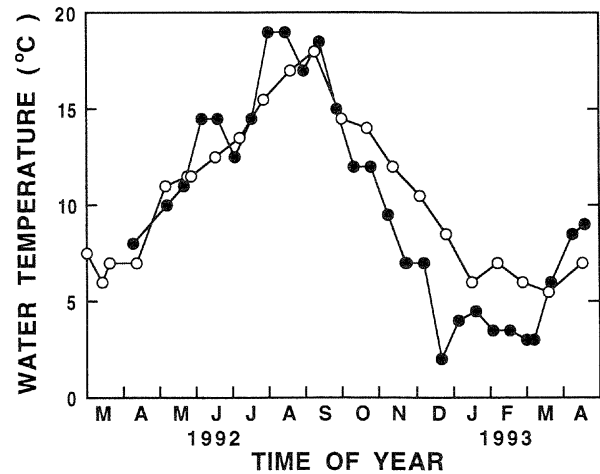


Fig. 1. Yearly fluctuations in water temperatures from March 1992 to April 1993 in the streams of Kyoto (closed circle) and Tokyo (open circle) where larval *H. kimurae* live.

Although the peak in egg laying in Tokyo was between early February and mid March, the water temperatures during this period ( $6$ - $7^{\circ}\text{C}$ ) were similar to corresponding temperatures recorded in Kyoto.

Increment in water temperatures was more rapid in Kyoto than in Tokyo; the water temperature reached to its maximum of  $19^{\circ}\text{C}$  in late July in Kyoto, but in Tokyo the maximum of  $18^{\circ}\text{C}$  was reached in early September. In Kyoto, the stream lower than the point of oviposition dried up during late October and late November, and larvae lost their habitat. With this decrease in water amount, water temperature more rapidly decreased in Kyoto than in Tokyo, where the amount of water was stable and the stream never dried up in the above three years.

### Development in Kyoto

Egg sacs in early developmental stages (St. 1-12) were found from late January to early April, with a peak between mid March and early April. Patterns of development from hatching to metamorphosis in larvae collected from the stream of Kyoto from May to September 1992 are shown in Fig. 2. As shown in the figure, hatching occurred at St. 41-42 from early April to mid May. The incubation period seemed to vary, but two pairs of egg sacs that were marked in early April hatched in mid May after about 40 days of incubation. Hatchlings stayed

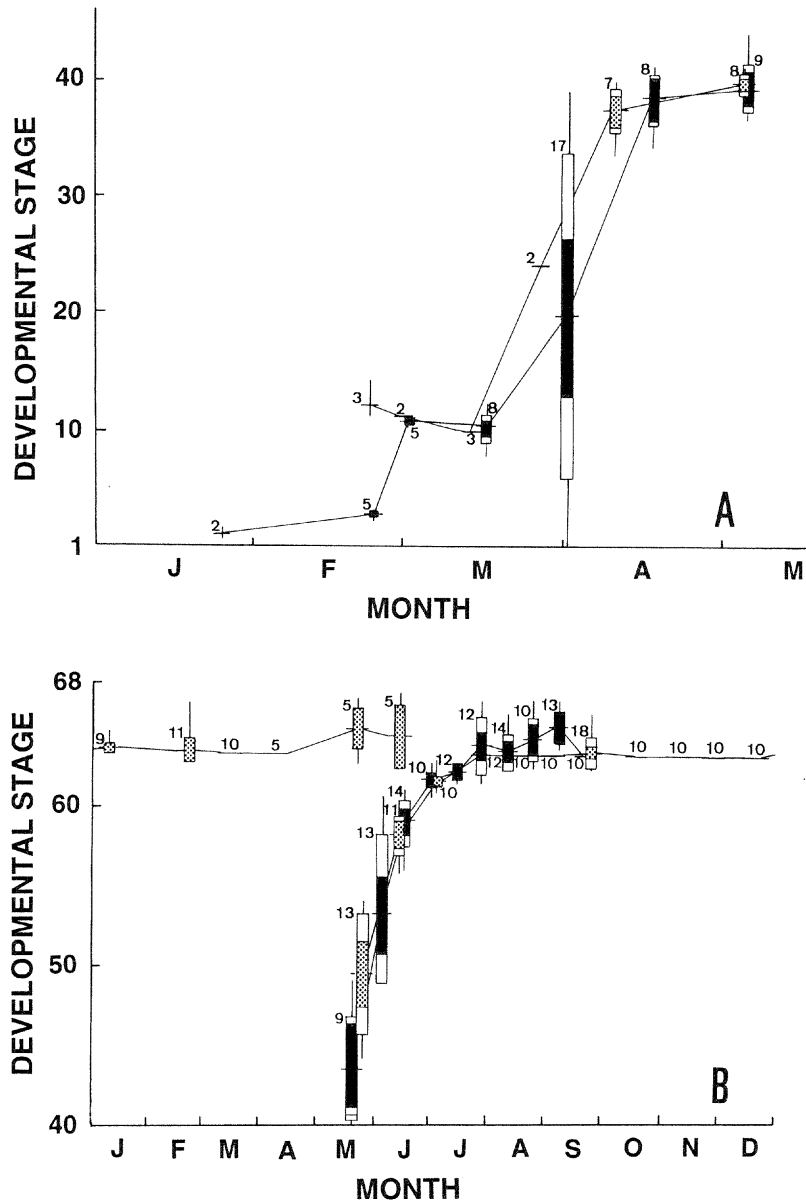


Fig. 2. Patterns of development in larval *H. kimurae* collected from the streams of Kyoto (closed rectangles) and Tokyo (hatched rectangles). (A) Development of larvae till mid May of the first year. (B) Development of larvae after mid May of the first year. Horizontal lines, vertical lines, open rectangles, and filled rectangles represent means, ranges,  $\pm$  SD, and  $\pm$  2SE, respectively. Letters represent sample sizes.

in the egg sac for a week before entering the stream, and apparently relied on yolk reserves.

In mid June, larvae reached St. 60 and were mostly observed in quiet pools rather than in swift currents. Fully developed larvae (St. 64) were first found in early July, and larvae found in late July or later were all at this stage or later. Metamorphosing individuals were found from late July to late September. No larvae were found in the stream in October or later.

#### *Development in Tokyo*

Freshly-laid egg sacs were found between early February and early April. Hatching occurred from early April to mid May. Embryos in a pair of egg sacs marked in early March of 1993

hatched about 60 days later.

Patterns of larval development, from hatching in May 1992 to metamorphosis in June 1993 are shown in Fig. 2. Larval growth in Tokyo was similar to that seen in Kyoto; most individuals reached St. 55 in early June and St. 60 in mid June. As in Kyoto, larvae were more often observed in quiet pools than in riffles.

Also as in Kyoto, development slowed after larvae reached St. 60, and some larvae reached full maturity (St. 64) in early July. After reaching mature stages, the developmental rate in the larvae from Tokyo decreased markedly compared to those from Kyoto. Of the larvae found in late September, 1992, only 21% (8/38) were undergoing metamorphosis; between October 1992 and March 1993 only about 2% were metamor-

phosing. Thus, most of larvae from Tokyo overwintered at St. 64 and metamorphosed between late May and mid June of the subsequent year; a few old larvae were observed in the stream after mid June. Mature larvae were seen in early July of 1992 in the latest case, and larvae that overwintered were never seen after mid July. This developmental pattern was observed every year during the survey period. On one occasion, smaller St. 52 larvae of the year were found eaten by overwintered older larvae (St. 64).

#### Larval growth

Increases in SVL from hatching to metamorphosis in larvae from Kyoto and Tokyo are shown in Fig. 3. Larval growth after hatching was less rapid in Kyoto than in Tokyo. After early July, when larvae reached mature stage, growth was slower in Kyoto than in Tokyo. That is, the larvae from Tokyo continued considerable growth in SVL even after this period until late November, and then ceased growing until late March; they resumed the growth in April.

#### Size at metamorphosis

Mean SVL ( $\pm 2$ SE) of metamorphosing (St. 65) to just metamorphosed (St. 68) larvae collected in Kyoto from early July to late September was  $20.4 \pm 0.76$  mm ( $n = 21$ ). Metamorphosing larvae collected in Tokyo in late September averaged  $29.5 \pm 2.22$  mm ( $n = 8$ ) in SVL, and were significantly larger ( $U$ -test,  $P < 0.01$ ). In the Tokyo population, larvae that metamorphosed within the year of hatching were significantly smaller at metamorphosis than larvae that overwintered ( $35.0 \pm 2.20$  mm,  $n = 8$ ;  $P = 0.01$ ).

## DISCUSSION

Because larval life history traits can influence post-metamorphic life history traits and adult fitness (e.g., Tilley,

1980; Smith, 1987; Semlitsch *et al.*, 1988), studies on the causes and consequences of such variations are crucial. The Cryptobranchoidea is regarded as primitive, key group in considering the evolutionary history of urodeles (Larson and Dimmick, 1993). Within the Cryptobranchoidea, the family Hynobiidae contains the most species that are either lentic or lotic breeders. Ecological aspects of a few members, such as lentic *Hynobius tokyoensis* (Kusano, 1981) and lotic *Onychodactylus japonicus* (Hayase and Yamane, 1982), have been well-studied, but no detailed study has been made on lotic members of the genus *Hynobius* as represented by *H. kimurae*.

We documented marked differences in growth and development of larvae at our two sites. This kind of variation is known in various members of tailed amphibians (e.g., Bruce, 1978, 1985; Semlitsch, 1983), but the pattern is not always identical. For example, Bruce (1978) found that the larval period of *Pseudotriton montanus* varies between populations, but because the growth rate in the larvae with shorter period is higher, body size at metamorphosis is nearly the same in the two populations, unlike the *H. kimurae* in this study. Interpopulation differences in growth rates of *P. montanus* studied by Bruce (1978) are considered to be the result of temperature differences, which does not seem to be so great for *H. kimurae* as discussed below.

The most marked environmental difference observed at the two localities was the stability of the water level in the stream; part of the stream was completely dried in Kyoto from October to November. Wilbur and Collins (1973) maintained that amphibians breeding in permanent habitats will vary considerably in the length of the larval period, but will metamorphose within a narrow size range, as contrasted with species adapted to temporary habitats, having necessarily brief larval lives and broader ranges of metamorphic size. Our results partly conform to this idea when the habitat in Kyoto is

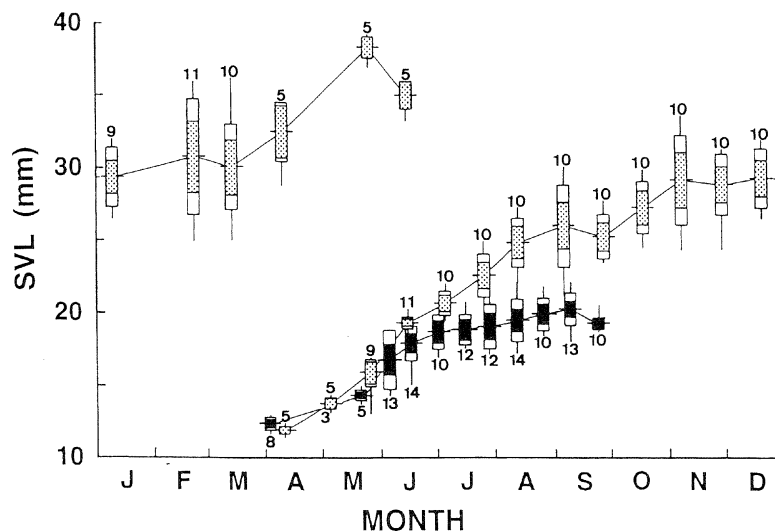


Fig. 3. Patterns of growth in SVL in larval *H. kimurae* collected from the streams of Kyoto (closed rectangles) and Tokyo (hatched rectangles). Horizontal lines, vertical lines, open rectangles, and filled rectangles represent means, ranges,  $\pm$  SD, and  $\pm$  2SE, respectively. Letters represent sample sizes.

regarded as a temporary one because part of the stream dries up, and that in Tokyo as a permanent one. In Tokyo, the length of the larval period varied considerably as stressed by Wilbur and Collins (1973), but the size at metamorphosis was variable and metamorphs in the first year were smaller than those of the second year. On the other hand, in Kyoto, larval period was brief, but the size at metamorphosis did not differ between larvae metamorphosed in late July and early September, and the degree of variation was very low (Fig. 3).

It is well-known that water temperature affects larval development in many amphibians (Duellman and Trueb, 1986), including lotic salamanders (Beachy, 1995). Much lower average winter and minimum temperatures in Kyoto might prohibit larval overwintering. The difference in the timing of metamorphosis in Kyoto and Tokyo, however, does not seem to be influenced by the difference in temperature conditions, because some larvae from Tokyo metamorphosed in the same season as larvae from Kyoto.

Food resource availability is also considered to affect larval growth and development in different *Desmognathus* populations (Austin and Camp, 1992). Interpopulation variation found in *H. kimurae* may be also affected by food resources which might be more sufficiently maintained in the Tokyo stream with more stable levels than in Kyoto.

In the stream of Tokyo, the water temperature was 6–7°C in January. Spawning of *H. kimurae* may be possible under these temperature conditions, but adult salamanders already aggregated near the breeding sites in December did not lay eggs until February or later. This behavior might be putting off the spawning season, and hence postponing the timing of larval hatching, and contributing to a decrease in the chance of preying on the first year hatched larvae by the overwintering, second year older larvae.

In Kyoto, some females laid eggs very early when water temperature was still very low. This may be related to the early metamorphosis in this population. Further, larval food availability, as well as the length of growth period, is considered important as a factor affecting interpopulation variation in egg size (Berven, 1982; Woodward, 1982; Takahashi and Iwasawa, 1988), and the occurrence of such variation suggests the possibility of differential food availability between Kyoto and Tokyo. Larvae from Kyoto may be obliged to complete growth and development in a shorter duration and probably with less food supply. Fewer but larger eggs with more yolk, laid by smaller females in Kyoto than in Tokyo may be related to this constraint; females may be allocating a limited amount of nutritional resources to each egg and thus enhancing the larval development.

Marked geographical variations are present in the adult body size of *H. kimurae* (Misawa and Matsui, unpublished data). In the two populations studied here, both sexes were smaller in Kyoto than in Tokyo. If the time required for sexual maturation is identical, the difference in the size at metamorphosis is considered to induce the difference in adult size (Juterbock, 1990). Thus, the difference in adult body size between Kyoto and Tokyo may be partly a result of differential

metamorphic size. It is also possible, though, that the period required for sexual maturity and/or the age structure may differ between the two populations and this may lead to the adult body size difference, as in *Desmognathus quadramaculatus*, whose age at metamorphosis and age at first reproduction are supposed to vary independently (Bruce, 1988).

In order to test the validity of speculations mentioned above, detailed examination of the other populations for the pattern of life histories should be pursued in addition to accumulation of additional data for the two populations, including larval food resource availability and age at the first reproduction. Such information could then be incorporated into clarification concerning causes and consequences of life history variations in *H. kimurae*.

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