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# Fluctuation in Abundance and Age Structure of a Breeding Population of the Japanese Brown Frog, *Rana japonica* Günther (Amphibia, Anura)

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**ABSTRACT**—A breeding population of *Rana japonica* was studied at a marsh on the campus of Hiroshima University in Higashi-Hiroshima during the five years 1995–1999. The mark-recapture study showed that the size of the breeding population varied from year to year, and increased more than twofold in 1999 in comparison with the preceding years. The sex ratio of the breeding population (male/female) was from nearly 1.0 to 1.6. Frogs of both sexes were estimated to breed for the first time at the age of one or two years, and their maximum age was four years according to skeletochronology using phalanges and mark-recapture. Modes of the estimated ages were one year for males during the study years except 1997, but one or two years for females. Two thirds of breeding frogs, irrespective of their sex, were estimated to breed only once throughout their lives.

**Key words:** *Rana japonica*, breeding population, skeletochronology, age structure, age at first reproduction

## INTRODUCTION

The Japanese brown frog, *Rana japonica*, is commonly distributed in Honshu, Shikoku and Kyushu of Japan (Maeda and Matsui, 1989). It breeds in still waters such as rice fields, marshes and small pools on rainy nights from January to April. Many field studies have been conducted on various aspects of life history of *R. japonica* such as breeding ecology, population dynamics, dispersal, growth, and conditions of habitats (Osada, 1968, 1978; Hasegawa, 1995, 1998; Fujioka and Lane, 1997; Mori, 1997; Kuramoto and Ishikawa, 2000; Tomioka, 2000; Osawa and Katsuno, 2001). But there have not yet been any reports on the age structures of breeding populations of *R. japonica*.

Age is one of the most important variables in elucidating population dynamics and life histories in long-lived animals such as amphibians. Skeletochronology is an effective method to determine the ages of amphibians, especially in temperate zones (Halliday and Verrel, 1988; Castanet and Smirina, 1990; Castanet *et al.*, 1993). Age structures, clarified by using skeletochronology, have been reported in some species of brown frogs, such as *R. temporaria* (Gib-

bons and McCarthy, 1984; Ryser, 1986, 1996; Augert, 1992; Augert and Joly 1993), *R. sylvatica* (Bastien and Leclair, 1992; Sagor *et al.*, 1998), *R. macrocnemis* (Tarkhnishvili and Gokhelaishvili, 1999), and *R. iberica* (Esteban and Sanchez, 2000). In Japan, only a few species or populations of anurans have been studied skeletochronologically (e.g., Kusano *et al.*, 1995 a, b; Khonsue *et al.*, 2001a, b).

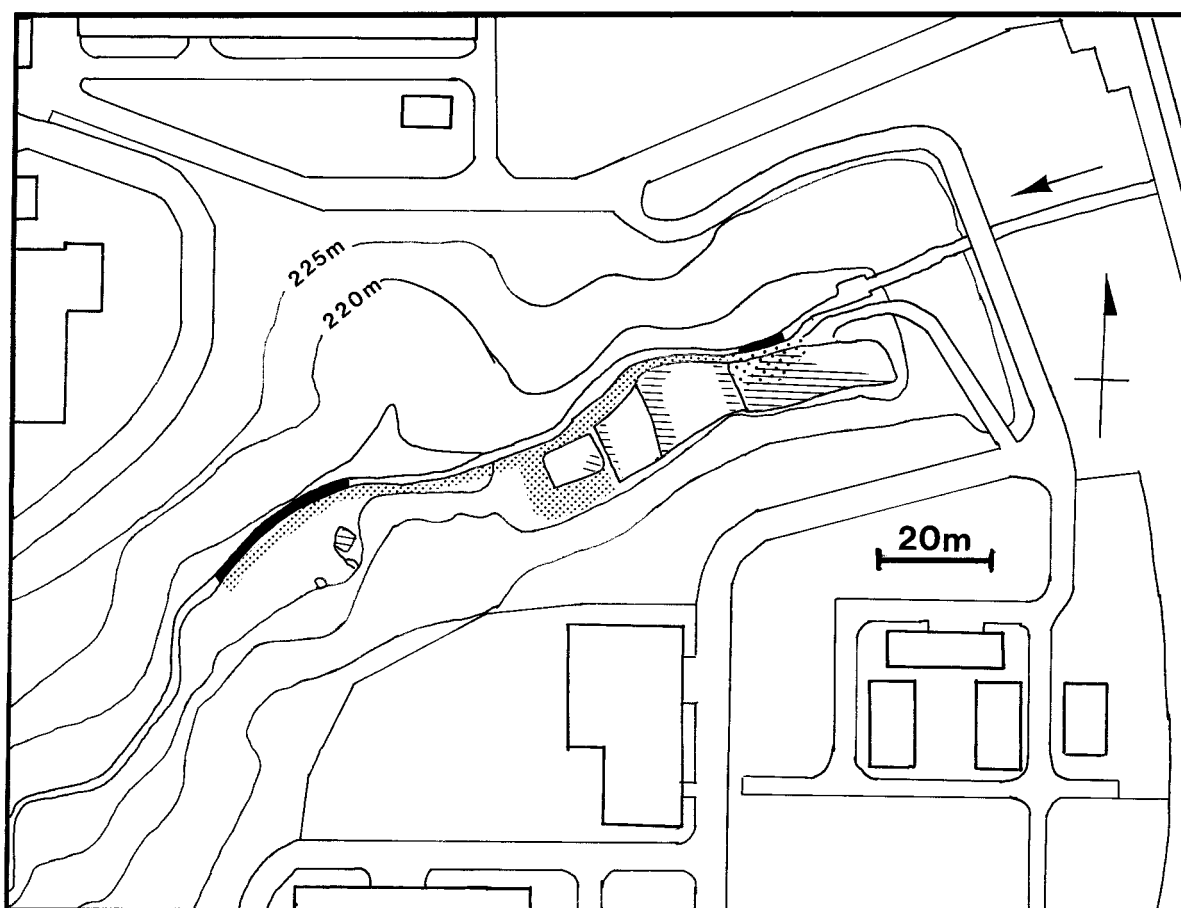
In the present study, we conducted a skeletochronological study to examine the age structure of a *R. japonica* population, and we also estimated abundance of the breeding population during five years by counting egg masses deposited and conducting mark-recapture studies. We report on the five-year fluctuation in size and age structure of the breeding population, and discuss the factors affecting the population dynamics.

## MATERIALS AND METHODS

We studied a population of *R. japonica* at the Hiroshima University Ecological Garden (34°24'N, 132°43'E, 220 m above sea level) located on the campus of Hiroshima University in Higashi-Hiroshima, Hiroshima Prefecture, Japan. The campus was constructed in an area of forests and farmland from 1978 to 1996. The Ecological Garden is a valley, about 1 ha in area, covered with the Japanese red pine, *Pinus densiflora*, and has been artificially managed since 1992 (Fig. 1). A marsh in the garden was made of aban-

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**Fig. 1.** Map of the study site. Striped areas show spawning sites of *Rana japonica*. Upper and lower solid areas in a stream show the area where the bank protection work was conducted in 1997 and 1998, respectively. Sparsely and densely dotted areas show paths and yards for construction materials in 1997 and 1998, respectively.

**Table 1.** Date of the bank protection work and the beginning of breeding of *R. japonica* in 1997 and 1998.

Year	Period of the construction work	Date of the first appearance of frogs		Date of the first spawning
		Male	Female	
1997	9 January–10 February	10 January	24 January	15 February
1998	6 January–2 March	8 January	3 February	3 February

done rice fields and is fed with water drawn from a nearby stream. *Rana japonica* has been breeding at the marsh since the breeding season of 1993. Some egg masses of *R. japonica* were also introduced to the marsh from a pool about 300 m from the garden in 1993 and 1994. In the study site, a construction project was undertaken to repair the stream banks during January–March in 1997 and 1998 (Fig. 1 and Table 1). There were not any direct disturbances on the spawning site, except that part of marsh was dried up or covered with iron plates to create paths and yards for construction materials at the beginning of the constructions. Therefore, the construction work did not damage directly egg masses spawned.

The mark-recapture study was conducted chiefly during the annual breeding season (January–March) and April of 1995–1999. In these periods, we visited the study site almost every day. We searched for breeding frogs in the marsh and the surrounding area at least once during each night. Frogs were captured by hand or using a dip net. Since 1996, the survey has also been conducted in autumn (September–November) in addition to the breeding sea-

son. When they were unmarked, they were measured for snout-vent length (SVL) to the nearest 1 mm with a slide caliper. Their sex and maturity were determined on the basis of secondary sexual characteristics, such as the development of thumb pads and ovaries. The frogs were individually marked by toe-clipping, and were released in the capture sites. Marked frogs were measured at the first capture in the season as well as unmarked ones. Group marking by toe-clipping was applied to newly metamorphosed juveniles in 1997–1998, since they were too small to be individually marked.

We determined the total number of breeding females by counting egg masses newly deposited every day, since a female *R. japonica* spawns a single egg mass during a season (Maeda and Matsui, 1989). The total number of breeding males was estimated from mark-recapture data using the program CAPTURE (see Otis *et al.*, 1978; White *et al.*, 1982; Thompson *et al.*, 1998). The traditional models of population estimation using mark-recapture data are difficult to apply to natural populations, because the crucial assumption that each animal has a constant and equal probability

of capture in each survey occasion is often violated. The program CAPTURE incorporates seven closed population models, allowing various relaxation of the assumption of equal catchability. These models allow for three major sources of variation in capture probabilities: time (t), behavioral responses (b), and heterogeneity among animals (h). We adopted a population estimate and its 95% confidence interval of the model that the program CAPTURE indicated the most appropriate, based on several goodness-of-fit tests among the seven models:  $M_0$ ,  $M_h$ ,  $M_b$ ,  $M_t$ ,  $M_{bh}$ ,  $M_{th}$ , and  $M_{tb}$ .

When an adult frog was captured, the fourth toe of the left or right hind leg was clipped off for skeletochronological study. Each toe was decalcified in 6% nitric acid for 40 min and washed in running tap water for 24 hr. After being embedded in paraffin, the second phalanx of each toe was sectioned (15  $\mu$ m thick) and stained with Lillie-Mayer's hematoxylin. We selected the best cross-section for each individual, and measured the major axes of the outer margin of the bone and the lines of arrested growth (LAGs) using an ocular micrometer attached to a light microscope (see Marunouchi *et al.*, 2000). For the specimens collected during January–April, we regarded the outer margin of the bone as an additional LAG, since any current year's growth had not yet occurred.

We assumed that the SVL and phalangeal sizes of individuals captured in autumn would be nearly equal to those in hibernation, because the mark-recapture study showed that most of the frogs apparently did not grow until the next spring. Therefore, for the analysis we pooled data for zero-year-olds captured in autumn, before their first hibernation, with data for one-year-olds captured in spring. Marunouchi *et al.* (2000) demonstrated that an individual's body size can be back-calculated accurately from the LAG diameter and the current body size using mark-recapture records in the population studied. The best valid back-calculation formula (BCF) is the Dahl-Lea method:  $L_i = L_c D_i / D_c$  ( $L$ : SVL in mm;  $D$ : bone diameter in  $\mu$ m;  $c$ : at capture;  $i$ : at  $i$ -th winter), and the absolute error of estimation was proved to be, on the average, only 2.3 mm. We collected a total of 195 juveniles in autumn and just after hibernation. Their SVLs ranged from 22 to 41 mm, with the mean being 30.7 (SD=3.4); the lower and upper limits of the 95% range based on the  $t$ -distribution were 24.0 and 37.4 mm, respectively. When SVLs back-calculated from LAGs were 37 mm or less, the frogs were estimated as juveniles at that time.

The significance level used in all tests was  $P=0.05$  (two-tailed). We adjusted  $P$ -values by the sequential Bonferroni procedure, when multiple comparisons or tests were performed (Rice, 1989).

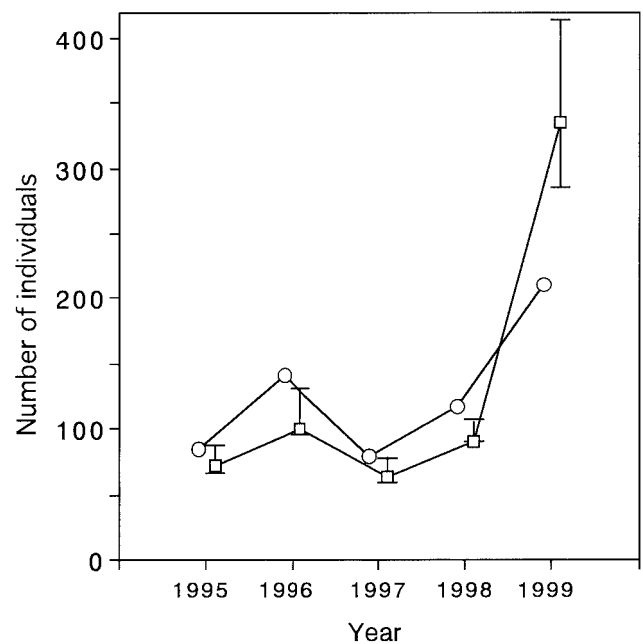
## RESULTS

### Population fluctuation and sex ratio

During the breeding season and April, the total number of captured frogs (the number of frogs recaptured at least once in parentheses) was 63 (45) in 1995, 100 (57) in 1996, 61 (30) in 1997, 89 (58) in 1998, and 194 (83) in 1999 for males, and 33 (7) in 1995, 49 (5) in 1996, 66 (11) in 1997, 77 (24) in 1998, and 90 (8) in 1999 for females. Based on the capture-recapture records of these males, the program CAPTURE presented population estimates using the most appropriate model with its 95% confidence intervals. For males, the recapture rate was high enough to give a good population estimates with sufficiently narrow confidence intervals, and the estimates approximated the total number of captured males in 1996, 1997, and 1998 (Fig. 2). For females, program CAPTURE failed to give a reliable population estimates: the estimates were six to ten times as large as the number of egg masses spawned with standard errors

larger than 1,000 except in 1998. We could not obtain a reliable population estimate for females using the capture-recapture records because of their pattern of emergence at the breeding site. The breeding population size of females, however, could be determined directly by counting a total number of egg masses deposited.

The breeding population studied fluctuated in abundance during the study period of five years (Fig. 2). Although the estimated male population was significantly smaller in 1996, 1997, and 1998 than the female population, the sex ratio (male/female) of the breeding population did not depart greatly from unity except in 1999. In 1999, population size increased abruptly and the sex ratio became biased toward males. In addition to 1999, 1996 saw an increase in the breeding population (especially females).



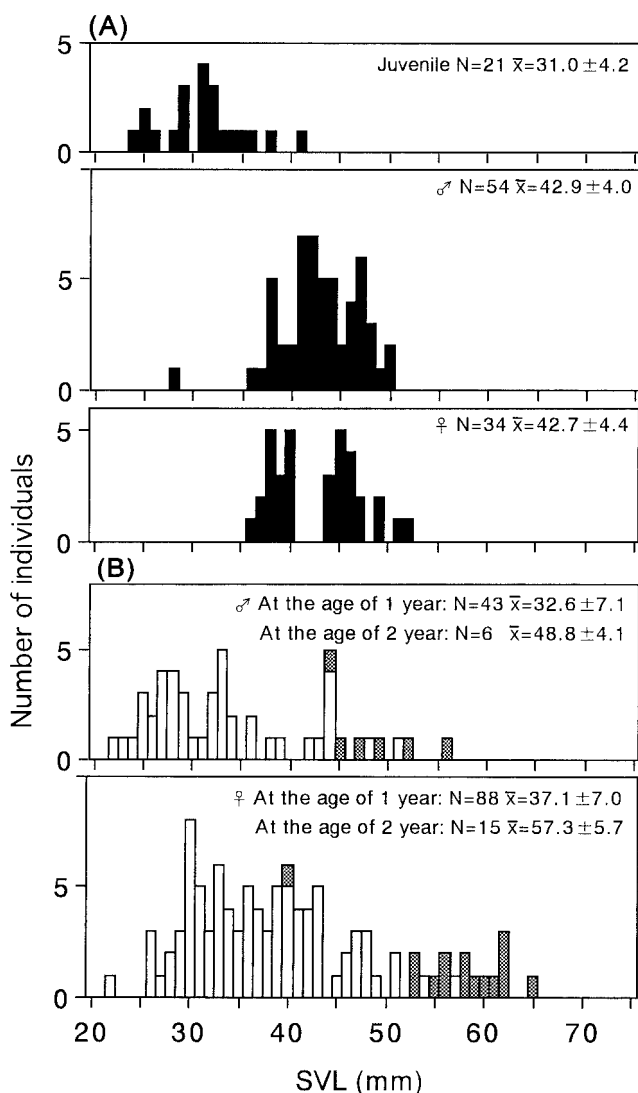
**Fig. 2.** Yearly change of the breeding population of *Rana japonica* from 1995 to 1999. Circles show abundance of females, estimated directly from the total number of egg masses deposited. Squares with vertical lines show abundance of males with 95% confidence interval, estimated using mark-recapture records (see text for details).

### Age determination

LAGs were clearly stained with hematoxylin and easy to count in phalangeal sections (see Marunouchi *et al.*, 2000). For many amphibian species, however, age estimation by skeletochronology has potential problems, such as endosteal resorption of periosteal bone, which may alter the early LAGs, especially at the age of one year. In the present study, of 63 males and 160 females showing LAGs, exclusive of periosteal, 24 males (38.1%) and 75 females (46.2%) showed partial resorption of the innermost LAGs. Therefore, we could not ignore the influence of endosteal resorption on age determination, and checked for this problem using the diameter of resorption lines measured.

To examine whether or not resorption lines had eroded completely the innermost LAGs, we compared the SVLs back-calculated from resorption lines with those of one-year-olds in the breeding season and April and zero-year-olds in autumn, ages of which were estimated by mark-recapture of newly metamorphosed juveniles (see Method section). These recaptured frogs included 21 juveniles, 54 males, and 34 females, and their SVLs ranged from 24 to 52 mm, and the upper limit of the range where 95% of them were included was 50 mm (Fig. 3A). We assumed that when the SVLs back-calculated from resorption lines were larger than 50 mm, the LAGs produced at the age of one year had been completely resorbed.

The SVLs back-calculated from resorption lines ranged

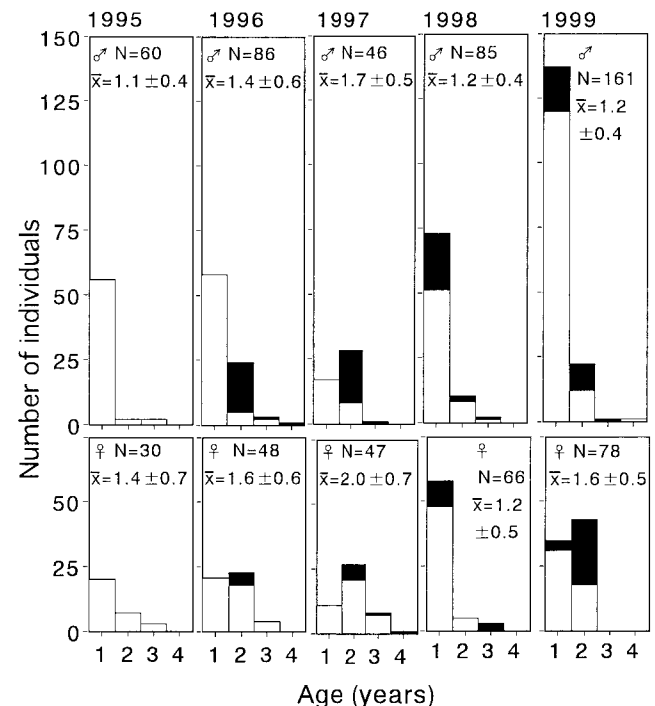


**Fig. 3.** Frequency distributions of SVLs of zero-year-old frogs captured in autumns and one-year-old frogs captured in breeding season and April of 1997–1999 (A) and back-calculated SVLs of frogs older than one year (B). In (A), solid bars show the SVLs of frogs that were aged by mark-recapture of newly metamorphosed froglets (see Fig. 4). In (B), Open bars and dotted bars show the back-calculated SVLs at the age of one and two years, respectively.

from 15 to 37 mm, with the mean being 24.1 (SD=3.8) for 411 males, and from 16 to 50 mm, with the mean being 26.9 (SD=5.1) for 286 females. We did not observe any resorption lines larger than the upper limit. Judging from the result, we concluded that complete resorption of the first LAGs had rarely occurred in our specimens, although the first LAGs of many individuals were partly destroyed.

### Standing age structure

The skeletochronological and mark-recapture studies showed that the age of adult *R. japonica* ranged from one to four years (Fig. 4). The modes of the ages were all one year for males except in 1997, and varied from one to two years for females. The age distributions differed significantly among years for both sexes (Kruskal-Wallis test,  $P<0.001$ ). Females were significantly older than males in 1999 (Mann-Whitney U-test,  $P<0.001$ ).



**Fig. 4.** Age structure of the breeding population of *Rana japonica*. Upper and lower figures show males and females, respectively. Age determination was made using skeletochronology (open bars) and mark-recapture (solid bars).

### Age at first reproduction

Frogs group-marked after metamorphosis in 1997 and 1998 were recaptured as juveniles or adults from their first autumn to the next April (Fig. 3A). This finding showed that not all one-year-olds began to breed.

Past SVLs were back-calculated from the diameters of the LAGs in frogs older than one year (Fig. 3B). For both sexes, these estimates at the age of one year coincided well with the actual SVLs of the juveniles and adults recaptured from their first autumn to the next April (Fig. 3A, B). In frogs

older than two years, SVLs back-calculated from the second LAGs were all larger than 37 mm for both sexes. Therefore, the back-calculated SVLs and the mark-recapture records indicated that the ages at first reproduction of both sexes were one or two years.

### Survival and breeding frequency

We determined the total number of frogs in each age class by age at first reproduction in each year, using the estimated yearly population size and the relative frequency of age class and age at first reproduction ( $N_{ij}$ ;  $N$ : the number of frogs;  $i$ : age;  $j$ : age at first reproduction). Then we rearranged these frequencies to make the temporal age structure of sequential cohorts (Table 2).

The temporal age structures of sequential cohorts showed that the modes of ages were two years ( $N_{1,1} < N_{2,1} + N_{2,2}$ ) in 1994 and 1997 cohorts for females. In individuals that began to breed at the age of one year, the abundance of two-year-olds was smaller than that of one-year-olds ( $N_{1,1} > N_{2,1}$ ) in all cohorts for both sexes.

In the 1994–1997 cohorts, we roughly estimated annual survival rates as  $N_{2,1}/N_{1,1}$ , and the ratio of two-year-olds in adult recruitment as  $N_{2,2}/(N_{1,1} + N_{2,2})$ .

Annual survival rates ranged from 8.7% to 39.7% for males, and from 23.5% to 57.7% for females (Table 2). Pooled data of the 1994–1997 cohorts indicates that females had significantly higher annual survival rate (47.5%) than males (30.5%) (Fisher's exact test,  $P < 0.001$ ).

As described above, all frogs matured sexually within two years of age, but the proportion of frogs that matured early or late varied from cohort to cohort. The ratio of late

matured frogs (the ratio of two-year-olds in adult recruitment) ranged from 8.2% to 25.8% for males, and from 22.7% to 41.8% for females (Table 2). Pooled data of the 1994–1997 cohorts indicated that females matured significantly later than males did (Fisher's exact test,  $P < 0.001$ ).

The lifetime breeding frequency (LBF) was estimated for each breeding adult as the number of breeding seasons that the frog experienced throughout life. It ranged from 1 to 4 for both sexes, and pooled LBF for the 1994–1995 cohorts showed that the mean was 1.4 (SD=0.5,  $N=152$ ) for males and 1.4 (SD=0.6,  $N=183$ ) for females, and did not differ significantly between both sexes (Mann-Whitney U-test,  $P=0.938$ ). And the ratios of adults that bred only once throughout their lives were 65.8% for males and 66.1% for females.

In the cohorts of 1995–1998, we could not detect any significant correlations between the number of egg masses spawned and the number of newly matured frogs in the next season,  $N_{1,1}$  (males,  $r=0.069$ ,  $P=0.931$ ; females,  $r=-0.677$ ,  $P=0.323$ ).

### Influence of constructions

In the study sites, construction work was conducted in the winters of 1997 and 1998. The population studied may have suffered some damages from the construction work, although it never damaged directly egg masses spawned.

The 1994 cohort had not experienced the construction work at the breeding site at the age of one and two years. To evaluate the effect of the construction work, pairwise comparisons of annual survival rates were conducted between cohorts by Fisher's exact test (one-tailed), using

**Table 2.** Temporal age structure and age at first reproduction of sequential cohorts of *R. japonica*.

Sex	Cohort	Age	1	2	3	4	Annual Survival rate	Ratio of two-year-olds in adult recruitment			
		Age at first reproduction	1	1	2	1			2	1	2
Male	1992		—	—	—	2	0	1	0		
	1993		—	0	2	0	3	0	0		
	1994		67	23	6	1	0	0	0	0.343	0.082
	1995		68	27	11	0	2	0	2	0.397	0.139
	1996		23	2	8	0	2	—	—	0.087	0.258
	1997		78	20	26	—	—	—	—	0.256	0.250
	1998		285	—	—	—	—	—			
	Total of 1994–1997		236	72	51	—	—	—	—	0.305	0.178
Female	1992		—	—	—	9	0	0	0		
	1993		—	17	3	9	3	2	0		
	1994		57	27	41	6	8	0	0	0.474	0.418
	1995		62	23	23	2	4	0	0	0.371	0.271
	1996		17	4	5	0	0	—	—	0.235	0.227
	1997		104	60	55	—	—	—	—	0.577	0.346
	1998		95	—	—	—	—	—	—		
	Tota of 1994–1997		240	114	124	—	—	—	—	0.475	0.341

**Table 3.** Estimation of overwintering survival of breeding adults. The number of survivors in the next breeding season was estimated by dividing the number of frogs recaptured by estimated catchability in the season (see text for details).

Sex	Year	No. captured in autumn	No. recaptured in the next breeding season	Estimated No. survivors in the breeding season	Overwintering survival rate
Male	1996–97	10	6	6	0.600
	1997–98	74	33	34	0.459
	1998–99	68	38	66	0.970
Female	1996–97	23	2	2	0.087
	1997–98	61	19	29	0.475
	1998–99	69	18	42	0.609

the 1994 cohort as a control group. We could not detect any significant reduction of survival for both sexes (all  $P$ 's > 0.200) except for the 1996 male cohort ( $P = 0.041$ ).

Frogs captured in autumn were recaptured in the next breeding season and April. Based on the mark-recapture data, we estimated the overwintering survival of breeding adults using estimated catchability in the breeding season (Table 3): we determined the total number of frogs that had survived to the next breeding season by dividing the number of recaptured frogs in the breeding season by estimated catchability in the season (the number of frogs captured/yearly population size), and estimated survival rates during the overwintering season (the number of survivors in the breeding season/the number of frogs captured in autumn). We compared the overwintering survival rate in the seasons of 1996–1997 and 1997–1998 with that in the 1998–1999 season as a control. The overwintering survival rate was significantly lower in the seasons of 1996–1997 and 1997–1998 for males (Fisher's exact test, one-tailed, all  $P$ 's < 0.005), and in the 1996–1997 season for females ( $P < 0.001$ ).

This result suggested that the construction work affected overwintering mortality of breeding frogs, especially males.

## DISCUSSION

The breeding population of *R. japonica* fluctuated from year to year during the five-year study period. Some important factors affecting the population dynamics of amphibian species have already been reported, e.g., desiccation of spawning sites (Berven, 1990; Friedl and Klump, 1997; Tarkhnishvili and Gokhelasvili, 1999), predation and cannibalism on larvae (Kusano, 1981; Baker, 1999), and predation during hibernation periods (Shirose and Brooks, 1997). In the present study, since water was artificially drawn from the nearby stream, the spawning site never dried up completely during the study period.

A crayfish, *Procambarus clarkii*, is considered to be one of the most important predators in the population studied. The crayfish was artificially introduced into the study site in 1993. It does not only prey on the eggs and larvae of *R. japonica*, but also can attack the breeding frogs even at

water temperature as low as 6°C, (Marunouchi, personal observation). The crayfish may have affected the dynamics of the breeding population studied, although we have not yet obtained any lines of quantitative evidence.

The magnitude of population fluctuation revealed by the present study was similar to the results of other studies conducted in relatively good habitats without any disturbance of breeding sites, such as farmland consolidation or abandonment of cultivation of rice fields (Osada, 1978; Mori, 1997; Hasegawa, 1998; Tomioka, 2000). In the present study, however, the bank protection work was undertaken in 1997 and 1998 (Fig. 1 and Table 1). Compared with 1996, the abundance of breeding frogs decreased in 1997 and increased slightly again in 1998. The increase rate was, however, smaller than the rates of other years.

Since adults were easier to capture near the breeding site in autumn than in summer, we think that adult frogs gathered near the breeding site before hibernation. So we think that the construction work may have affected the abundance of breeding frogs. Reduced survival of the breeding frogs also suggests the possibility that the construction work actually affected the dynamics of the breeding population (Tables 2 and 3). The constructions never dug up directly bottom of the marsh where frogs hibernated (Marunouchi, personal observation). But the constructions may have disturbed frogs hibernating around the marsh, because *R. japonica* also hibernates in terrestrial environment, such as under fallen leaves and trees on the surface of soil (Osada, 1968; Hiba Society of Natural History, 1996).

Yearly fluctuations in age structure have been reported in some species of anurans: e.g., *R. temporaria* (Gibbons and McCarthy, 1984; Ryser, 1986; Augert and Joly, 1993), *R. macrocnemis* (Tarkhnishvili and Gokhelasvili, 1999), *Hyla arborea* (Friedl and Klump, 1997), and *Bufo fowleri* (Green, 1997). The age structure of *R. japonica* also fluctuated from year to year. Therefore, it is difficult to estimate the past abundance based on an estimated survival schedule, as Ryser (1986) did.

In general, animals allocate a large amount of energy to reproduction. Therefore, their growth is greatly retarded after they reach sexual maturity (e.g., Kusano, 1982; Ryser, 1989). A smaller interval between two LAGs indicates

retarded growth, and the first of these smaller intervals may indicate the age at sexual maturity (but see Augert and Joly, 1993). In *R. japonica*, Marunouchi *et al.* (2000) showed that in the population studied, some individuals' growth rates remained high even after sexual maturity. Therefore, in the present study it was difficult to estimate age at sexual maturity based on only the growth pattern of LAGs, unlike the case in other studies (Francillon-Vieillot *et al.*, 1990; Augert, 1992; Augert and Joly 1993; Kusano *et al.*, 1995a, 1995b). For *R. japonica* in the population studied, back-calculation of past SVLs is an effective way to estimate age at first reproduction, as Marunouchi *et al.* (2000) demonstrated.

In a stable population, the age structure should show a fairly steady decline from the maximum in the youngest age until the oldest age classes. The present study showed that the modal age of breeding frogs was often two years, which was not the minimum age at first reproduction. The standing age structures of the breeding population (Fig. 4) suggested the possibility that the relative frequency of a specific cohort became larger than the relative frequencies of others (Gibbons and McCarthy, 1984; Friedl and Clump, 1997). The temporal age structures of sequential cohorts (Table 2) showed another possibility that not all individuals matured simultaneously at a specific age (Hemelaar, 1988; Augert, 1992; Miaud *et al.*, 1993). The coexistence of juveniles and adults from their first autumn to the next April (Fig. 3A) supported the latter possibility for *R. japonica*, as in other studies (Ryser, 1988; Forester and Lykins, 1991; Castanet *et al.*, 1996; Khonsue *et al.*, 2001b).

For both sexes of *R. japonica*, about two thirds of breeding adults bred only once throughout life. Such a low lifetime breeding frequency may increase extinction risk in a small, isolated population if the population suffers complete reproductive failure in consecutive years (Osada, 1978; Hasegawa, 1998; Tomioka, 2000). In the present study, the abundance of one-year-old adults did not correlate with the total number of egg masses spawned during the previous breeding season. Yearly fluctuation of the adult population may be due largely to variation in juvenile recruitment (Berven, 1990), and therefore the most important factor affecting the dynamics of the breeding population may be mortality during the larval stage. To understand fully the mechanisms of population dynamics of *R. japonica*, further analyses should be conducted on larval and juvenile stages of this population.

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