

Adult Sex Ratio of a Population of the Japanese Salamander, *Hynobius tokyoensis* (Caudata: Hynobiidae)

TAMOTSU KUSANO^{1*} AND MASAFUMI INOUE²

¹ Department of Biological Sciences, Graduate School of Science and Engineering, Tokyo Metropolitan University, Minami-ohsawa 1-1, Hachioji-shi, Tokyo 192-0397, JAPAN

² Kyoto Kagaku Co. Ltd., Shimotoba 35-1, Watarise-cho, Fushimi-ku, Kyoto 612-8393, JAPAN

Abstract: Adult sex ratio is considered to be a key factor in understanding sexual selection, mating behavior, life history and population dynamics. Following Fisher (1930), a general consensus was formed that sex ratio tends to be largely balanced. However, gainsaying this, large variations in sex ratio have been reported among populations and species of amphibians. There is little information on sex ratio in natural populations of Japanese hynobiid salamanders. To help fill this gap, we conducted a long-term census of a population of *Hynobius tokyoensis* from 1976 to 1985 and estimated the adult sex ratio (the proportion of males within the population) at 0.578 from data gathered during eight non-breeding seasons. The bootstrap 95% confidence interval was 0.516–0.641. The study showed that the adult sex ratio was significantly biased toward males in the population studied; it was nearly 1.5:1 (males:females). Age at first reproduction is 1 year later in females, which suggests that the most likely factor driving the unbalanced sex ratio is the differential maturation rate between the sexes.

Key words: Adult sex ratio; Male-biased ratio; Population-level sex ratio; Differential maturation rate; *Hynobius tokyoensis*

INTRODUCTION

From an ecological point of view, adult sex ratio is considered to be a key factor in understanding sexual selection, mating behavior, life history and population dynamics. A general acceptance of Fisher's (1930) argument for the evolutionary stability of equal investment in the sexes of offspring led to a general acceptance that adult sex ratios tend to be largely balanced and thus may have dampened inter-

est in researching them (see Hardy, 2002; Donald, 2007). However, in amphibians, adult sex ratio varies from species to species depending on reproductive mode, mating system and overall distribution of resources (Zug et al., 2001). Moreover, large intraspecific variations have been reported both temporally and geographically (e.g., Arntzen, 2002; Salvido, 2008). Although a significant departure from an equal sex ratio has sometimes been observed in adult populations of urodelan species (for example, in *Desmognathus* [Organ, 1961], *Eurycea* [Bruce, 1988], *Lissotriton* [Griffiths, 1984], and *Ambystoma* [Beneski et al., 1986]), our knowledge of adult sex ratios in these

* Corresponding author. Tel: +81-42-677-1111
ext. 3761; Fax: +81-42-677-2559;
E-mail address: tamo@tmu.ac.jp

species is still less than adequate. A possible explanation for this lack of knowledge is that estimating adult sex ratios from a single or even several field samples is unreliable, because males and females may behave differently or have different rates of activity, especially in the breeding season, and consequently may have different chances of being detected and captured (Donald, 2007; Salvido, 2008).

We conducted a long-term census of a breeding population of the Japanese salamander, *Hynobius tokyoensis*, during both breeding and non-breeding seasons. We estimated adult sex ratio from the 8-years worth of census data during non-breeding seasons and found a significant male-biased sex ratio. Herein, we report on this study and discuss factors that could drive this bias in sex ratio.

MATERIALS AND METHODS

Study site

Hynobius tokyoensis is a typical lentic breeding salamander that inhabits the Kanto district and a part of Fukushima Prefecture, Japan. Ecological characteristics of *H. tokyoensis*, such as population dynamics and various life history parameters, have previously been published (Kusano, 1980, 1981, 1982; Kusano and Miyashita, 1984; Kusano et al., 2006). A breeding population of *H. tokyoensis* was studied in Habu (35°44'N, 139°14'E), Hinode-machi, Tokyo, about 35 km west of the Tokyo city area. The study site is located in a hilly region, 200–300 m asl. The hill-side consists predominantly of artificial coniferous plantation, *Cryptomeria japonica* and *Chamaecyparis obtusa*, and secondary deciduous forest. There are many small ponds and abandoned paddy fields at the foot of the hill. The salamanders use some of these ponds and flooded paddy fields as breeding sites. A part of one hill slope (including a small pond and a paddy field), was selected as the study area (see Kusano and Miyashita, 1984).

Field census

The field census was carried out approxi-

mately once a week from May–November (which corresponds with the non-breeding season), from 1976 to 1985 inclusive, with the exception of 1982 and 1983. We searched for the salamanders for about 2 hours during daylight, following a fixed route. Sites where salamanders were relatively easy to detect were selected, with the search route arranged to include all sites and cover as wide an area as possible (Kusano and Miyashita, 1984). Salamanders were found at the entrance of burrows, under rocks, leaf litters, layers of moss and decaying logs. Specimens were measured for total length and snout-vent length (from the tip of the snout to the posterior end of the cloacal opening; SVL) with a sliding caliper, and sexed on the basis of the status of secondary sexual characters, such as morphology of the cloacal region, throat coloration and ovisac development (Sato, 1943; Hasumi, 2001; Ishihara, 2008). The minimum SVLs of breeding adults were 50 and 55 mm for males and females, respectively (Kusano, 1982; Kusano et al., 2006). Individuals which did not show any sign of secondary sexual characteristics and were smaller than 55 mm in SVL were classified as juveniles. All captured individuals were released at the capture points after they were marked by toe-clipping. However, juveniles with a SVL of less than 35 mm could not be marked, due to their short digits.

We only used data from first captures to avoid replication of data caused by the same individual being captured more than once (Hurlbert, 1984).

Statistical analysis

We used the software R 2.13.1 (R Development Core Team, 2011) for statistical analyses. In accordance with Wilson and Hardy (2002), the adult sex ratio was expressed as the proportion of mature males to the total population of mature males and mature females. We conducted logistic regression analysis to test the effect of yearly data on sex ratio (Crawley, 2007). The standard error and 95% confidence interval of sex ratio was estimated by bootstrap analysis under stratified resampling

with year as strata, based on 9999 replicates, and we applied the percentile method to estimate the confidence interval (Davison and Hinkley, 1997; Rizzo, 2008). Departure from an equal sex ratio was assessed by whether or not 0.5 fell within these confidence intervals. The significance level used in all tests was $P < 0.05$.

RESULTS

During the study period of eight non-breeding seasons, we captured salamanders on the forest floor a total of 150, 102, 5, and 51 times for males, females, unsexed adults, and juveniles, respectively. We captured a total of 129 males and 94 females.

The annual sex ratio of the captured adults varied from 0.421 to 0.810, and the mean was 0.593 (SD=0.110). The sex ratios were scattered around 0.6 in most years (Fig. 1). The logistic regression analysis showed that there were no significant differences in sex ratio with respect to year (likelihood ratio test; $\chi^2 = 7.703$, $df = 7$, $P = 0.360$). In view of this, we estimated the adult sex ratio of this population

to be 0.578, using the pooled data as a basis.

The standard error of the sex ratio was estimated at 0.033 by bootstrap analysis, and the 95% confidence interval was 0.516–0.641. This interval failed to include 0.5, so there was a significant departure from an equal sex ratio. This result showed that in the population studied, the adult sex ratio was biased significantly toward males: the sex ratio was nearly 1.5:1 (males:females) rather than 1:1.

DISCUSSION

Adult sex ratio is one of the most important life-history variables for understanding and simulating the dynamics of salamander populations in the field. Although more than 20 species of hynobiid salamanders inhabit Japan, very little data on their sex ratios have been documented (e.g., Hasumi and Kanda, 2007). Population studies of hynobiid species have been conducted mainly during breeding seasons, because many breeding adults aggregate in limited small areas and can be captured easily (Kusano, 1980, 1982; Misawa and Matsui, 1999; Ento and Matsui, 2002). However, because females appear in the breeding site just before spawning and stay there for only a few days, catching females is more difficult than catching males (see Kusano, 1980, 1982; Kusano et al., 2006). As a result, sex ratio data from a breeding season survey tend to be both highly male-biased and unreliable.

In contrast, we have not yet found any reports suggesting that one sex is more difficult to catch than the other during the non-breeding season. Hence, the adult sex ratio may be best estimated from census data gathered during the non-breeding season. However, gathering data during the non-breeding season has its own difficulties. It is not easy to capture a sufficient number of adults during this season, due to the secretive nature of their habits on the forest floor after post-breeding dispersal (Kusano and Miyashita, 1984). A long-term census was, therefore, needed to gather data to reliably estimate adult sex ratio. The study reported herein showed a male-

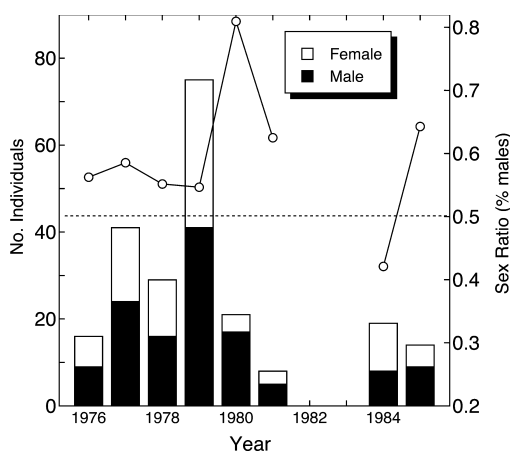


FIG. 1. Yearly variation in sex ratio of adult individuals captured on the forest floor during non-breeding seasons. Open circles indicate the proportion of mature males to the total population. Solid and hollow histograms indicate the number of males and females captured, respectively. The horizontal dotted line indicates an equal sex ratio (0.5).

TABLE 1. Adult sex ratio of salamanders and newts.

Family	Species	Country	Adult sex ratio		Literature
			% males	Direction of bias	
Salamandridae					
	<i>Notophthalmus perstriatus</i>	USA	0.44	female-biased	Johnson (2002)
	<i>N. viridescens</i>	USA	0.64	male-biased	Massey (1990)
			0.67	male-biased	
	<i>Triturus cristatus</i>	Sweden	0.56	male-biased	Hagström (1979)
		UK	0.47		Verrell and Halliday (1985b)
	<i>Triturus (=Lissotriton) helveticus</i>	UK	0.25	female-biased	Harrison et al. (1983)
	<i>T. (=L.) vulgaris</i>	UK	0.45	female-biased	Bell (1977)
			0.40	female-biased	Verrell and Halliday (1985a)
			0.33	female-biased	Griffiths (1984)
			0.28	female-biased	Harrison et al. (1983)
		Sweden	0.50		Hagström (1979)
Plethodontidae					
	<i>Desmognathus fuscus</i>	USA	0.61	male-biased	Organ (1961)
	<i>D. moniticola</i>	USA	0.49		Organ (1961)
	<i>D. ochrophaeus</i>	USA	0.55	male-biased	Organ (1961)
		USA	0.46		Tilley (1980)
			0.55		
	<i>D. quadramaculatus</i>	USA	0.46		Organ (1961)
	<i>D. wrighti</i>	USA	0.62	male-biased	Organ (1961)
	<i>Eurycea wilderae</i>	USA	0.63	male-biased	Bruce (1988)
	<i>Speleomantes strinatii</i>	Italy	0.57	male-biased	Salvidio (2008)
Ambystomatidae					
	<i>Ambystoma jeffersonianum</i>	USA	0.71	male-biased	Douglas (1979)
	<i>A. macrodactylum</i>	USA	0.63	male-biased	Beneski et al. (1986)
	<i>A. texanum</i>	USA	0.60	male-biased	Petranka (1984)
			0.63	male-biased	Williams et al. (2009)
	<i>A. tigrinum</i>	USA	0.75	male-biased	Williams et al. (2009)
Hynobiidae					
	<i>Onychodactylus fischeri</i>	Russia	0.50		Solkin (1993)
	<i>Hynobius tokyoensis</i>	Japan	0.58	male-biased	This study
	<i>Salamandrella keyserlingii</i>	Japan	0.68	male-biased	Hasumi and Kanda (2007)

biased adult sex ratio in a natural population of a hynobiid species, using long-term census data during the non-breeding seasons.

We conducted a literature search on the adult sex ratio of urodelan populations. These data and those from the present study are summarized in Table 1. The table shows that the adult sex ratio varies markedly according

to species and populations, from 0.25 (a highly female-biased sex ratio of *Triturus (=Lissotriton) helveticus* in mid-Wales) to 0.75 (a highly male-biased one of *Ambystoma tigrinum* in the USA). The adult sex ratio also seems to differ among families; populations of Salamandridae seem to be more female-biased, and those of Ambystomatidae are more male-

biased (Table 1). The sex ratio of the population studied here is moderately male-biased among urodelan populations.

Possible factors driving the above mentioned sex ratio biases in urodelan species may include: biennial reproduction for females (Anderson et al., 1971; Arntzen, 2002), sex ratio at birth (Sever and Dineen, 1978), differential maturation rate (Sever and Dineen, 1978; Salvidio, 2008), and differential mortality between the sexes (Organ, 1961; Bell, 1977; Verrell and Halliday, 1985a). Biennial reproduction can be excluded as a factor affecting sex ratio in this study, because sampling was conducted during non-breeding seasons. Further, we cannot discuss biased sex ratio at birth at present, because there is no information on biased sex ratio at birth in hynobiid species. Differential maturation rate between sexes would seem to be the factor likely to drive the significant bias in the adult sex ratio in the population studied.

The mean SVL of breeding adults is significantly larger in females than males in the study population (Kusano, 1982; Kusano et al., 2006). The minimum SVL is 48.5 mm and 55.5 mm for males and females, respectively. This sexual difference in body size suggests a differential maturation rate between the sexes. Kusano (1982), on the basis of growth rates of juveniles under laboratory conditions, mean SVL at metamorphosis, and the minimum SVL of breeding adults in the field, suggests that age at first reproduction is delayed 1 year in females. In fact, a skeletochronological study showed that the youngest breeding individuals were 4 years old among males, but 6 years old among females (Kusano et al., 2006), although we failed to detect any significant differences in the mean and median ages, perhaps because of the small sample size for the females. The annual survival rate of metamorphosed individuals has been estimated to be nearly 0.7 (Kusano, 1982). Taking this into account, the delayed sexual maturity of females can increase the sex ratio to up to 0.588 ($1.0/(1.0+0.7)$), if the age at first reproduction is delayed 1 year in females. This ratio

is very close to the observed value (0.578), which suggests that the difference in age at first reproduction according to sex is an important factor driving the male-biased sex ratio in the population studied.

Another possible factor is differential mortality between the sexes. If the biased sex ratio is due mainly to differential mortality, females will be shorter-lived than males. However, the skeletochronological study mentioned above did not detect any significant differences in the mean age of breeding adults between sexes (Kusano et al., 2006). In fact, we do not have sufficient data to evaluate the importance of the differential mortality between the sexes, at present. Long-term mark-recapture studies may be needed to determine the difference in mortality rate between the sexes.

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