RESEARCH ARTICLE

Age, Body Size, and Sexual Dimorphism in Size and Shape in *Salamandrella keyserlingii* (Caudata: Hynobiidae)

Masato Hasumi

Abstract In organisms with determinate growth, sexual size dimorphism (SSD) occurs before maturity during the developmental process of growing apart, an ontogenetic perspective on the evolution of SSD. If the direction of SSD (female-larger SSD) is known, patterns of growth can be tested with one-tailed statistical distributions. In indeterminate growing organisms as well, does SSD occur before maturity? If it occurs, whether is females' larger mean body size caused by a difference in age at maturity, age-specific size, divergent growth prior to maturity, or selection on post-maturational growth? How important is biphasic, sexual shape dimorphism (BSSD) for determinants of SSD? Biphasic characteristics are those that differ between adult aquatic- and terrestrial-phase morphs, and shape is size of a characteristic relative to body size. To address those questions, I determined age and body size based on a careful description of a growth trajectory for each sex in *Salamandrella keyserlingii*, using 555 independent data points from skeletochronological studies. Females reached maturity at 3–4 years of age, a year later than males that reached maturity at 2–3 years of age (mean body size: males = 57.63 mm, females = 61.70 mm; delayed sexual maturity resulted in SSD). However, SSD was highly detected before maturity (SSD index = 0.097), and females after maturity continued to grow and resulted in larger asymptotic size than males. Traits of BSSD were greater in males than in females. These results suggest that when determining SSD the difference in mean adult-body size results from the difference in age-specific size and the female-larger SSD develops to resolve intersexual ontogenetic conflict by allowing small-sized males to swell their whole body during the aquatic phase as much as large-sized females.
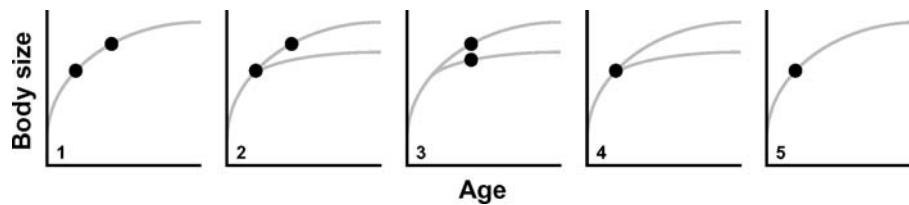
Keywords Age at maturity · Age structure · Growth trajectory · Indeterminate growth · Population structure · Skeletochronology

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Introduction

Body size is important in determining life-history traits for many organisms (Kirkpatrick 1984). In organisms with determinate growth, individuals cease growing after maturity (Badyaev 2002; McKenzie et al. 2007). In those with indeterminate growth, however, individuals continue to grow throughout their lives (Kozlowski and Uchmañski 1987; Halliday and Tejedo 1995). Growth in ectothermic vertebrate taxa such as fishes, amphibians, and reptiles is indeterminate, but the onset of breeding activities is accompanied by a sharp decrease in growth rate; age at maturity depicts a trade-off between fecundity, developmental time, and survival (i.e., energy allocation: Day and Taylor 1997; Heino and Kaitala 1999; Barot et al. 2004). Knowing the age structure of a vertebrate with indeterminate growth contributes to our understanding of its population demographic parameters from an ecological, developmental, ontogenetic, and evolutionary perspective (Bruce 1993; Olsson and Shine 1996; Pough et al. 2001).

If we can numerously sample variously aged individuals, we would be able to evaluate how well different asymptotic-growth models (Halliday and Tejedo 1995; Cox and John-Alder 2007; John-Alder et al. 2007) elucidate determinants of sexual size dimorphism (SSD). These models include five trajectories (Fig. 1): (1) males and

Fig. 1 Growth trajectory models in indeterminate growing vertebrates. Solid circles indicate sexual maturity in either sex. Sexual size dimorphism (SSD) is caused by a difference in (1) age at maturity, (2) age-specific size, (3) divergent growth prior to maturity, or (4) selection on post-maturational growth. SSD does not occur in Model 5. See text for the detail

females follow the same growth curve, but one sex matures later at a larger size than the other despite the same asymptotic size (this growth pattern can result in SSD); (2) males and females mature at different ages, both showing reduced growth after maturity, and one sex with delayed sexual maturity reaches a larger asymptotic size (SSD); (3) males and females follow different growth curves prior to maturity and mature at different sizes, in which age at maturity is similar (SSD); (4) males and females mature at the same age, but one sex continues to grow more than the other (SSD); and (5) males and females follow the same growth curve and mature at the same age (no SSD).

Model 4, which one sex continues to grow after maturation but the other does not, implies a difference in selection on post-maturational growth but not on growth before maturity. A similar implication occurs for Model 1. If selection acting on the growth on males and females results in changes in SSD, Models 2 and 3 will be the only ones consistent with selection on growth before maturity. These models apply to many mammal and bird species with determinate growth, neonate males and females of which are identical in size (Badyaev 2002; McKenzie et al. 2007). Adopting such an ontogenetic perspective on the evolution of SSD in indeterminate growing amphibians requires a careful description of growth trajectories for each sex. This process of 'growing apart' before maturity (Badyaev 2002) has been documented in reptiles, in which neonate males and females are the same size (Cox and John-Alder 2007; John-Alder et al. 2007).

If the direction of SSD (female-larger SSD) is known, patterns of growth can be tested with one-tailed statistical distributions. I investigated three specific questions related to amphibian SSD (Halliday and Tejedo 1995; Malmgren and Thollesson 1999; Salvidio and Bruce 2006). First, does SSD occur before maturity? Second, does a difference in mean adult-body size result from a difference in age at maturity that leads to a difference in age structure of a population? If males are the same size as females for any given age but tend to be younger, this trajectory coincides with Model 1. If a difference in age-specific size occurs (i.e., females grow more quickly than males so that they are larger at a given age), the data support Model 2. If divergent growth occurs prior to maturity (i.e., males and females grow at different rates before maturity, and females mature at a larger size than males with a similar age), the data support Model 3. Finally, if a difference exists in selection on post-maturational growth (i.e., females continue to grow after maturation but males do not), the data support Model 4. Third, how important is biphasic, sexual shape dimorphism (BSSD) for determinants of SSD? Biphasic characteristics are those that differ between adult aquatic- and terrestrial-phase morphs, and shape is size of a characteristic relative to body size (Hasumi and Iwasawa 1990). To address these questions, I generated data on lines of arrested growth (LAG) for age estimation in *Salamandrella keyserlingii* Dybowski, 1870, using monthly samples from spring to fall over three consecutive years (Hasumi and Kanda 2007).

Methods

Study Animal and Area

Salamandrella keyserlingii has the broadest range of any amphibian species worldwide (~ 12 million km^2: Duellman and Trueb 1986), extending from eastern Europe through subarctic Siberia, including the northern portions of Kazakhstan, Mongolia, China, North Korea, and Japan (Kuzmin 1994; Borkin 1999). I conducted this study in a wetland at Otanoshike (part of Kushiro Marsh) located outside the Kushiro Shitsugen National Park (26,861 ha), Kushiro-shi, Hokkaido Prefecture, a northern island of Japan (43°01′ N, 144°18′ E; 4 m elevation). Detailed description of this wetland appeared in Hasumi and Kanda (1998, 2007). Although Kushiro Marsh is of geologically recent origin (3000–5000 years old), transformed from a cove into which the sea had intruded (Scott 1991), *S. keyserlingii* is considered a relict of the Würm glacial epoch (20,000 years ago) and to have previously been distributed around the periphery of this cove. Because the study area I selected was located at this periphery, it could be regarded as a naturally distributed, ancient population. Also, this population corresponded to the southern limit of distribution for *S. keyserlingii* among all populations within Kushiro Marsh (i.e., southernmost population).

Monitoring Techniques

On 27 and 28 May 1995, I set a grid of 160 pitfall traps in a 100×55 m area (for the detail, see Hasumi and Kanda 2007). I placed moist sphagnum moss on the bottom of each trap to reduce desiccation of captured animals and covered traps with plastic lids to prevent access of animals between census periods. I opened traps during the terrestrial phase of the nonbreeding season (late May–October) from 1995 to 1997 with 7–13 days opened each month for a total of 141 days. I also opened traps during the April–May breeding season in 1996 (22 days) and 1997 (34 days). I inspected traps every morning throughout the census periods. During the April–May breeding season in 1995 (15 days), I captured aquatic-phase adults by dip netting at night from a fen. I collected a total of 673 individuals: 528 captures (525 measured and three unmeasured) and 105 recaptures from the traps and 30 captures and 10 recaptures from the fen.

I weighed each individual (body mass: BM) to within 0.05 g, using a beam balance. I measured the broadest head width (HW), maximum tail height (TH), snout–anterior vent length (SAVL: distance from the tip of the snout to the anterior angle of the vent), snout–posterior vent length (SPVL: from the tip of the snout to the posterior angle of the vent), and tail length (TL: from the posterior angle of the vent to the tip of the tail) to within 0.01 mm, using digital solar calipers, by a modification of Wise and Buchanan's (1992) method without using anesthesia. I recorded age class, sex, and visual characteristics such as throat coloration, development of visible ovisacs, and dorsal color pattern for each individual, according to Hasumi (2001b). These data were used to categorize all individuals into five classes: adult males, adult females, unsexed individuals, juveniles, and metamorphs defined as individuals that completed metamorphosis within the last month. I used SPVL to distinguish unsexed individuals (SPVL $\geq$ 52.00 mm) from juveniles (SPVL $<$ 52.00 mm) by applying the minimum SPVL for known adult females (52.00 mm). That is, I defined unsexed individuals as an individual without sexual characteristics but with larger body size than the smallest adult female, which could not be assigned to either sex.

In capture–mark–recapture (CMR), I marked sala manders individually using up to one toe clip per appendage (i.e., nonadditive toe-clips) and released them either to a breeding fen or near the pitfall traps where they were captured. I used natural deformities in lieu of clipping toes, without removing the deformities. As needed, I renewed toe-clips of recaptured salamanders using the individual characteristics recorded previously for identification.

Skeletochronology and its Background

I fixed toes, clipped from each individual from CMR study, in 10% neutral buffered formalin in situ. I exploited a normal toe to prevent underestimation of the number of LAGs caused by toe regeneration and conducted all skeletochronological procedures according to Hasumi and Watanabe (2007). Their method provides rapid and accurate processing of very tiny, phalangeal bones, resulting in up to 100% reading success in age. One LAG reflects one period of arrested growth such as hibernation and, in rare cases, aestivation (Francillon-Vieillot et al. 1990). I counted the number of LAGs and estimated practical age as

$$a + (b - c)/365,$$

where 'a' $=$ number of LAGs and 'b' $=$ Julian date of capture. The parameter 'c' was applied by each of three Julian dates of the completion of breeding for 1995–1997 (118, 129, and 148, respectively by year: Hasumi and Kanda 1998). The parameter 366 was an alternative to 365 in the 1996 estimation because of leap year. The 3-year dataset included 555 individuals.

Halliday and Verrell (1988) discussed four methods for determining the age of amphibians and reptiles: CMR, extrapolation from size-frequency data, skeletochronology, and testis lobation, a method applicable to only males of salamander species having multiple testis-lobes that increase in number with age. They concluded that only skeletochronology and CMR are reliable. In amphibians, CMR usually is not very effective for age estimation because of a small number of recaptured animals (Marvin 2001, 2009; Blackwell et al. 2003). In addition, nonindependence of recapture data restricts the analysis of a relationship between age and body size (Griffiths and Brook 2005). Thus, within a recent decade, techniques for age determination in that taxon have converged on the most accurate predictor of age, skeletochronology, in which LAGs are counted in bone tissues, although inaccuracies in age estimation may occur because of endosteal resorption of the innermost LAG (Eden et al. 2007).

In many skeletochronology papers, adult specimens are sampled for the LAG detection only once from aquatic-breeding sites, and metamorphs are sampled around there. It thus has induced the absence of multi-year juveniles prior to maturity. If numerous specimens are sampled not only once during the aquatic phase of the breeding season but also many times during the terrestrial phase of the breeding and nonbreeding seasons (often spanning 7–8 months from spring to fall, except for a winter-dormant period), a more precise estimation of age is possible (Kozlowski and Uchmañski 1987). It is likely difficult to capture multi-year juveniles prior to maturity, as well as postbreeding adults of migratory salamanders (e.g., ambystomatids, hynobiids,

salamandrids) because these adults emigrate upland and retreat to subterranean burrows or under cover objects above ground and thus cannot be readily found (Verrell and Davis 2003; Hasumi and Kanda 2007; Hasumi et al. 2009). Such methodology may then be impractical for the investigator.

Statistical Analysis

To ensure independence of data, I included only data on first capture for analysis ($n = 555$). Body size (SPVL) ranged from 23.11 to 72.33 mm and was partitioned into 50 frequency categories of one mm each. I used chi-square tests to compare size-frequency distributions between groups to see if they differed in population structure and to compare male to female age structure. I compared mean age between sexes with the Aspin-Welch test for unequal variances. I tested normality of a size-frequency SPVL distribution for each cohort with the D'Agostino-Pearson normality test. I calculated the SSD index (Lovich and Gibbons 1992) as

$$a/b - 1,$$

where 'a' = mean SPVL of larger sex and 'b' = mean SPVL of smaller sex. I assigned this index a positive value when females were the larger sex and a negative value when males were the larger sex. To detect development of SSD, I compared SPVLs of males and females before and after the modal year at maturity (males = 3, females = 4) using a one-way analysis of variance (ANOVA) with each sex as categorical effects of interaction. I tested for significant differences in morphometric variables (i.e., $\log_{10}$-transformed BM, HW, TH, and TL) between adult classes (BSSD: aquatic-phase males vs. terrestrial-phase males; SSD: terrestrial-phase males vs. terrestrial-phase females) with multivariate analysis of covariance (MANCOVA) using $\log_{10}$-transformed SPVL as the covariate. After conducting MANCOVA, I compared body size characteristics with analysis of covariance (ANCOVA) using $\log_{10}$-transformed SPVL as the covariate for body shape and pairwisely with a post-hoc Fisher's PLSD for body size, to detect whether these characteristics had an equal quantification between males and females.

I calculated a nonlinear, sigmoid growth equation between age (x-axis: years) and SPVL (y-axis: mm) with a quasi-Newton method (Davidon-Fletcher-Powell algorithm: Zeleznik 1968), an algorithm without any constraints (Tarling and Cuzin-Roudy 2003). The starting point of the growth curve was the time at metamorphosis and growth during the aquatic larval stage was not considered (Hemelaar 1988; Arntzen 2000). The growth coefficient k, the rate at which maximum size is approached (asymptotic maximum size: $SPVL_{max}$), defines the shape of the curve (Charnov 1993). I calculated the parameters $SPVL_{max}$ and k from the definitive growth curve. I calculated 95% support-plane confidence intervals for $SPVL_{max}$ and k and considered differences according to treatment to be significant when these confidence intervals did not overlap (Dunham 1978). In comparison, I fitted a modified Bertalanffy's (1938) equation according to Hemelaar (1988) where age at metamorphosis (birth) was added to growth parameters (also see Arntzen 2000; hereafter called 'Bertalanffy-Hemelaar') because of its widespread use in amphibian studies. I compared growth parameters between complete data (i.e., data on 0-year metamorphs, multi-year juveniles, unsexed individuals, and adult males or females) or reliable data (excluding data on unsexed individuals from complete data) and deficient data (data on 0-year metamorphs and either sex), in which the null hypothesis was that parameter estimates of growth models fitting the complete or reliable data were not different from those fitting the deficient data. I used least squares regression to test for a relationship between age (x-axis: years) and SPVL (y-axis: mm) for each cohort. I tested a difference in regression slopes between sexes with ANCOVA. All significance levels were tested at $\alpha = 0.05$ (two-tailed).

Methodological Considerations

For many growth trajectory studies, theoretical flaws have been overlooked in evaluating an ontogenesis of SSD. Using Bertalanffy's (1938) equation under indeterminate growth fails to account for the change in energy allocation at maturity and should be incapable of accounting for pre- and post-maturity growth (Day and Taylor 1997). This equation provides a good description of somatic growth after maturity but does not do so prior to maturity (Lester et al. 2004). It may be applied as a phenomenological description of indeterminate growth pattern (Czarnoleski and Kozlowski 1998). In amphibians with biphasic life cycles, confusion exists between Bertalanffy's (1938) equation and the Bertalanffy-Hemelaar (1988) equation (e.g., Miaud et al. 1999, 2000; Tsiora and Kyriakopoulou-Sklavounou 2002; Kyriakopoulou-Sklavounou et al. 2008); the former does not initially include the growth parameter, age at metamorphosis (birth). Confusion also occurs with the exponential function to a value x, $\exp(x)$ or e^x (i.e., confused as $\exp^x$: Miaud et al. 2001; Olgun et al. 2001). No growth trajectory can be described using this function.

Although many skeletochronological studies are affected by the growth trajectory problems mentioned above, leading to false modeling, a growth curve for each sex appears to fit Bertalanffy's (1938) equation or the Bertalanffy-Hemelaar (1988) equation when using data on 0-year metamorphs and multi-year juveniles in nonmigratory salamanders such as ovoviviparous salamandrids and terrestrial plethodontids (Miaud et al. 2001; Olgun et al. 2001; Leclair et al. 2006) and monthly samples in neotropical frogs (Marangoni et al. 2009). In contrast, because data on

0-year metamorphs are included in the estimation of growth curves for migratory frogs or salamanders, without data on multi-year juveniles, it is impossible to fit nonlinear models (e.g., Caetano and Castanet 1993; Miaud et al. 1999, 2000). For example, male and female *Ichthyosaura* (formerly *Mesotriton*) *alpestris* reach maturity at 11 and 9 years old, respectively, and data on juveniles of 1–8 years old or more are lacking (Miaud et al. 2000). Other growth curves fail to be fitted by nonlinear models because of using only adult data (e.g., the starting point of the growth curve is erroneously set at zero, contrary to the indication of considering the metamorphic size of 20 mm as the starting point for Bertalanffy's equation: Tsiora and Kyriakopoulou-Sklavounou 2002; Kyriakopoulou-Sklavounou et al. 2008).

Results

Body Size Distribution

Population structure analysis revealed, in general, two distinct groups in the overall histogram with sexes and age classes pooled (Fig. 2). Metamorphs were separated from other individuals ($\chi^2 = 443.303$, df = 49, $P < 0.0001$), showing a discontinuous SPVL distribution with a first small peak from 30 to 31 mm ($n = 15$). Partial overlap of the SPVL distribution from 37 to 40 mm was observed between metamorphs and juveniles. A size-frequency SPVL distribution from 46 to 52 mm overlapped between juveniles and adult males, resulting in the absence of a partition of the SPVL distribution. A second large peak of SPVL ($n = 40$), mainly composed of adult males ($n = 28$) followed by adult females ($n = 11$), occurred in the modal size from 59 to 60 mm.

Size-frequency SPVL distributions showed skewness for all individuals ($n = 555$, $K^2 = 86.757$, $P < 0.0001$) and unsexed individuals ($n = 23$, $K^2 = 16.543$, $P = 0.0003$). However, these distributions did not deviate from normality for metamorphs ($n = 63$, $K^2 = 5.199$, $P = 0.0743$), juveniles ($n = 63$, $K^2 = 3.684$, $P = 0.1585$), juveniles plus unsexed individuals ($n = 86$, $K^2 = 4.577$, $P = 0.1014$), adult males ($n = 276$, $K^2 = 2.103$, $P = 0.3494$), and adult females ($n = 130$, $K^2 = 0.853$, $P = 0.6527$). A normal distribution in the juveniles plus unsexed individuals suggested that they could be categorized as one size-class (i.e., indication of complete data that included unsexed individuals for depicting growth equations was rational because these individuals could not be separated from juveniles in their size-frequency distribution).

Body Size Comparison

A difference in minimum SPVL and BM between males (46.21 mm and 2.50 g) and females (52.00 mm and
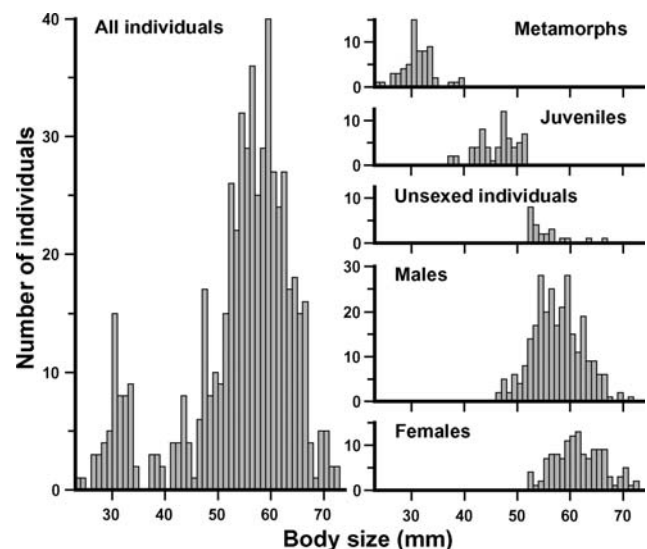
Fig. 2 Size-frequency distributions in body size (SPVL) within the range of 23.11–72.33 mm (50 categories every one mm) for 555 individuals: 63 metamorphs (N), 63 juveniles (J), 23 unsexed individuals (U), 276 adult males (M), and 130 adult females (F). Because of small body size with a discontinuous SPVL distribution, N is clearly separated from other individuals. Large individuals (≥52.00 mm SPVL) other than M and F are categorized as U

3.20 g) suggested that body size at maturity was different according to sex (Appendix 1). Metamorphic minimum SPVL was 23.11 mm (BM = 0.45 g). Ontogenetic SSD development was detected with a high significance before and/or after maturity (Table 1).

Dimorphic traits were detected in both the aquatic and terrestrial phases (Table 2). Aquatic-phase males had greater body size and shape in all parts of the body (BM, HW, TH, and TL) than terrestrial-phase males, except for BM relative to body size. In respect of the size, BM and HW were greater in terrestrial-phase females than in terrestrial-phase males. In contrast, males had longer tails than females. For the shape as well, TL was greater in terrestrial-phase males than in terrestrial-phase females, despite the influence of the larger SPVL of females (i.e., TL was a sexually dimorphic trait in both size and shape during the terrestrial phase). Thus, these differences in size and shape quantified males and females equally.

Lines of Arrested Growth

In each phalangeal bone sectioned transversely, LAGs were detected clearly (Hasumi and Watanabe 2007: Fig. 1B). Endosteal resorption of the innermost LAG (Eden et al. 2007) was not observed, and thus the periosteal bones only were counted. Inside collapse of bones occurred frequently at a 2- or 3-year ring, indicating a sharp growth of bones. Outside LAGs were dense on and after a 2- or 3-year ring for males and a 3- or 4-year ring for females.

Table 1 Ontogenetic SSD development before and/or after maturity

Maturity	Sex	n	SPVL (mm)			SSD index	F	df	P
			Mean	SE	Range				
Before	M	82	53.98	0.39	46.21–60.86	0.097	63.802	1,125	<0.0001
	F	45	59.24	0.54	52.00–68.06				
After	M	194	59.17	0.30	46.64–71.52	0.064	16.968	1,277	<0.0001
	F	85	63.00	0.49	52.11–72.33				
All	M	276	57.63	0.28	46.21–71.52	0.071	68.881	1,404	<0.0001
	F	130	61.70	0.40	52.00–72.33				

Body size (SPVL) was compared between males (M) and females (F) before and/or after the modal year at maturity (M = 3, F = 4) with a one-way ANOVA

Table 2 Multivariate analysis of covariance (MANCOVA) and analysis of covariance (ANCOVA) of $\log_{10}$-transformed body mass (BM), head width (HW), tail height (TH), and tail length (TL) between adult classes (BSSD: aquatic-phase males [Ma] vs. terrestrial-phase males [Mt]; SSD: Mt vs. terrestrial-phase females [Ft]), using $\log_{10}$-transformed snout–posterior vent length (SPVL) as the covariate, and their pairwise comparisons with a post-hoc Fisher's PLSD

| ANCOVA					Fisher's PLSD	
Variable	SS	F	P	Difference	P	Difference
Ma vs. Mt						
BM	0.001	0.596	0.4408	Ma = Mt	<0.0001	Ma > Mt
HW	0.115	287.25	<0.0001	Ma > Mt	<0.0001	Ma > Mt
TH	0.226	252.069	<0.0001	Ma > Mt	<0.0001	Ma > Mt
TL	0.036	29.592	<0.0001	Ma > Mt	<0.0001	Ma > Mt
MANCOVA: Wilks' $\lambda = 0.312$, $F_{4,270} = 149.178$, $P < 0.0001$						
Mt vs. Ft						
BM	0.002	0.781	0.3775	Mt = Ft	<0.0001	Mt < Ft
HW	0.011	29.056	<0.0001	Mt < Ft	<0.0001	Mt < Ft
TH	0.041	39.303	<0.0001	Mt < Ft	0.1305	Mt = Ft
TL	0.24	193.23	<0.0001	Mt > Ft	<0.0001	Mt > Ft
MANCOVA: Wilks' $\lambda = 0.602$, $F_{4,370} = 61.098$, $P < 0.0001$						

No LAG was observed in 63 metamorphs, indicating that they did not suffer an arrested growth between birth and capture.

Age at Maturity

A typical distribution of chi-square values in age structure revealed that males reached maturity at 2–3 years old (modal year = 3: $\chi^2 = 528.149$, df = 275, $P < 0.0001$) and females at 3–4 years old (modal year = 4: $\chi^2 = 182.207$, df = 129, $P = 0.0029$; Fig. 3). Mean age was 3.96 years for males ($n = 276$, range [minimum age at maturity–maximum longevity] = 1.92–9.99, SD = 1.38) and 4.78 years for females ($n = 130$, range = 2.94–7.92, SD = 1.19). Males were younger on average than females ($t = 6.133$, df = 291, $P < 0.0001$). When including 115 recapture data, maximum longevity was 10.37 years for males and 8.09 years for females.

A sigmoid growth equation was fitted between age and SPVL in each sex (Fig. 4). For 63 metamorphs, mean age and SPVL were 0.35 years and 31.18 mm, respectively. Using complete data, a Bertalanffy-Hemelaar equation between age (x-axis: years) and SPVL (y-axis: mm) was $y = 65.72 - (65.72 - 31.18) \exp(-1.341 \times (x - 0.35))$ for males and $y = 71.38 - (71.38 - 31.18) \exp(-1.129 \times (x - 0.35))$ for females. A positive linear relationship was found between age and SPVL in each cohort (Fig. 4), excluding 0-year metamorphs ($n = 63$, $R^2 = 0.034$, $P = 0.1446$). When deficient data (i.e., excluding data on
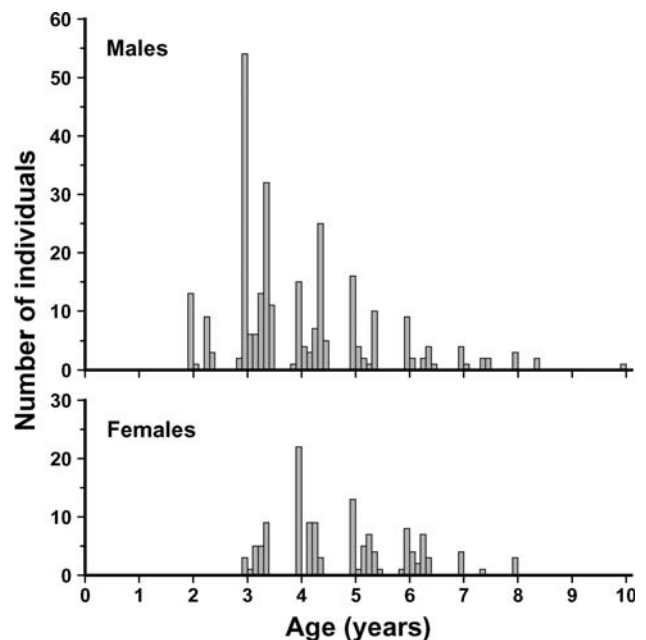
Fig. 3 Age-frequency distributions for 276 adult males and 130 adult females (every 0.1 years). Males and females reach maturity at 2–3 years old (modal year = 3) and 3–4 years old (modal year = 4), respectively. Minimum age at maturity–maximum longevity are 1.92–9.99 years for males and 2.94–7.92 years for females. When including 115 recapture data, maximum longevity is 10.37 years for males and 8.09 years for females
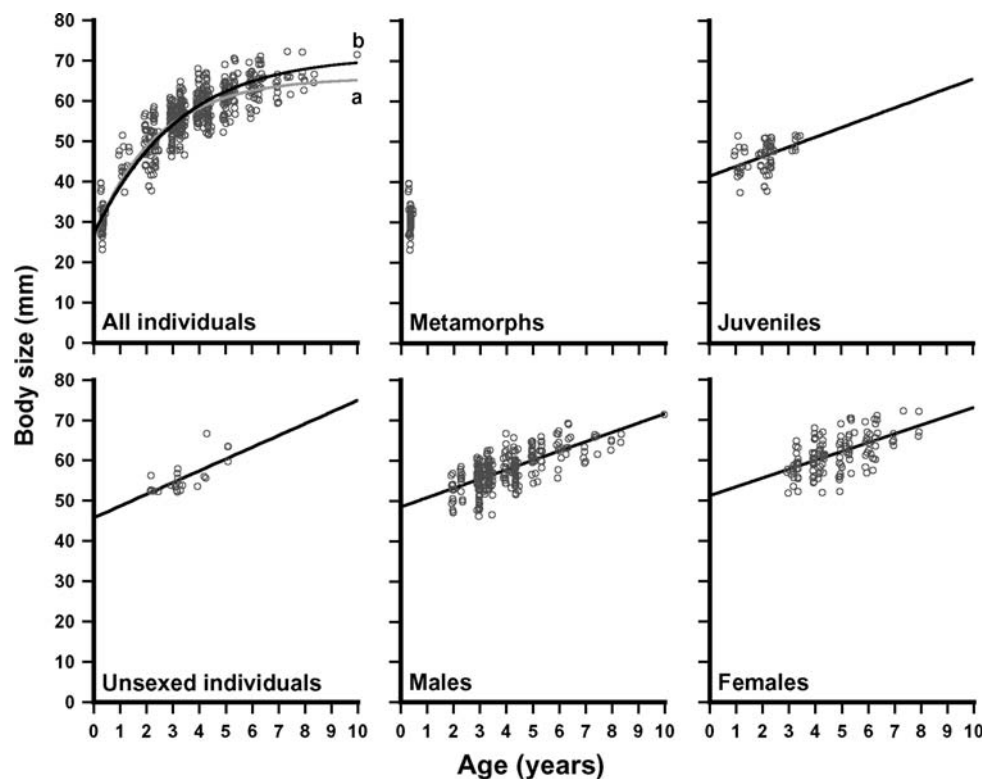
Fig. 4 Nonlinear, sigmoid growth equations between age (x-axis: years) and body size (SPVL [y-axis: mm]) with all individuals (276 adult males [M], 130 adult females [F], 23 unsexed individuals [U], 63 multi-year juveniles [J], and 63 0-year metamorphs [N]): (a) males (MUJN): $y = 2972.74 \times ((\exp(0.418768 \times (x + 11.9603)) - 1)/ (\exp(0.418768 \times (x + 11.9603)) + 1)) - 2907.02$ ($n = 425$, $R^2 = 0.8712$, $SPVL_{max} = 65.72$, $k = 1.341$); and (b) females (FUJN): $y = 379.038 \times ((\exp(0.327477 \times (x + 8.47830)) - 1)/(\exp(0.327477 \times (x + 8.47830)) + 1)) - 307.655$ ($n = 279$, $R^2 = 0.9032$, $SPVL_{max} = 71.38$, $k = 1.129$). Logistic or exponential growth equations have lower regression coefficients (R^2) than those of sigmoid growth equations. A positive linear relationship is found between age and SPVL in J ($R^2 = 0.181$, $P = 0.0005$, y intercept = 41.379, slope = 2.415), U ($R^2 = 0.465$, $P = 0.0003$, y intercept = 45.785, slope = 2.913), M ($R^2 = 0.479$, $P < 0.0001$, y intercept = 48.481, slope = 2.312), and F ($R^2 = 0.323$, $P < 0.0001$, y intercept = 51.274, slope = 2.183). Regression slope is greater in M than in F with female-larger body size at y intercept ($SS = 396.319$, $F_{1,403} = 32.609$, $P < 0.0001$)

multi-year juveniles [$n = 63$] and unsexed individuals [$n = 23$] from complete data) were used to depict a growth trajectory for males, regression coefficient (R^2) and growth coefficient (k) were overestimated, each with a greater value than when all individuals or all but unsexed individuals were included, and vice versa asymptotic maximum size ($SPVL_{max}$) was underestimated with a smaller value than when all individuals were included (Table 3). Among these parameters, differences in growth coefficient (k) between complete or reliable data and deficient data were significant. A similar trend occurred for females.

Discussion

Ectothermic vertebrate taxa such as fishes, amphibians, and reptiles exhibit indeterminate growth that implies a continuous but reduced increase in body size after maturity. Thus, sex-specific ontogenesis and age at maturity may elucidate proximate determinants of SSD (Bruce 1993;

Lester et al. 2004; Cox and John-Alder 2007). In anurans, Monnet and Cherry (2002) showed that variation in SSD can be explained in terms of differences in age structure between sexes in breeding populations (i.e., Model 1 is applicable). Unlike such recent suggestions for the importance of a difference in age structure, a difference in age-specific size may be concerned with the development of SSD, as well as that in divergent growth prior to maturity (Cox and John-Alder 2007; John-Alder et al. 2007). In the population of *S. keyserlingii* studied herein, females reached maturity at 3–4 years of age, a year later than males that reached maturity at 2–3 years of age. Although males were younger on average than females, a different growth curve for each sex and 'other related results' (see below) clearly indicated that males matured earlier than females when growth rate was similar, females before maturity grew more quickly than males that showed reduced growth for the change in energy allocation, and females after maturity continued to grow and resulted in larger asymptotic size than males (i.e., Model 2 is applicable).

Table 3 Comparison of regression coefficient of each growth equation (R^2) and growth parameters ($SPVL_{max}$ and k) $\pm$ 95% planar support confidence intervals (95% CI) between three treatments (when using complete, reliable, and deficient data) in males' class (MUJN, MJN, and MN) and females' class (FUJN, FJN, and FN)

Class	Treatment	n	R^2	$SPVL_{max} \pm$ 95% CI	$k \pm$ 95% CI
Males	MUJN	425	0.8712	65.72 $\pm$ 2.96	1.341 $\pm$ 0.060
	MJN	402	0.8745	65.89 $\pm$ 3.92	1.328 $\pm$ 0.079
	MN	339	0.9065	64.19 $\pm$ 2.17	1.550 $\pm$ 0.052
Females	FUJN	279	0.9032	71.38 $\pm$ 8.00	1.129 $\pm$ 0.126
	FJN	256	0.9074	69.75 $\pm$ 4.95	1.409 $\pm$ 0.100
	FN	193	0.9416	67.68 $\pm$ 4.67	1.443 $\pm$ 0.099

Differences in parameter estimates were considered significant if there was no overlap in associated 95% CI. M: adult males; F: adult females; U: unsexed individuals; J: multi-year juveniles; and N: 0-year metamorphs

This sexual difference was positively supported by dense LAGs outside of a 2- or 3-year ring for males and a 3- or 4-year ring for females, as documented in *Hynobius tokyoensis* (Kusano et al. 2006). Data on age structure showed better fit to population structure with size-frequency distribution for females, in which body size was larger in females than in males in a given size. These data also provided growth trajectories with an ontogenesis of SSD detected before maturity (SSD index = 0.097) and with a nearly full range of practical ages between years, reflecting monthly growth from April to October except for a winter-dormant period, for our understanding of the indeterminate growth pattern (Kozlowski and Uchmañski 1987). This pattern displays rapid growth prior to maturity, which results in the best growth curve being sigmoid (Arntzen 2000; Blackwell et al. 2003; Marvin 2009).

Female-larger SSD may therefore depend on fecundity that controls offspring production, according to energy allocation or reproductive investment (Heino and Kaitala 1999; Marvin 2009). In terms of a functional fecundity, selecting larger body size in *S. keyserlingii* results in more increased clutch size (averaged 200 eggs in a full clutch: Hasumi and Kanda 1998) than that of other caudate amphibians (e.g., cryptobranchids, amphiumids, and some ambystomatids lay 100 eggs in a clutch; Salthe 1969) and more delayed age at maturity in females than in males (this study). This perspective on age and size can well explain proximate determinants of SSD in amphibians. An alternative explanation for female-larger SSD is that *S. keyserlingii* may be under a phylogenetic constraint or represent an ancestral trait at maturity and may not be responding to selection (Bruce 2000).

The aforementioned results support the concept of growing apart, an ontogenetic perspective on the evolution

of SSD (Badyaev 2002). Besides, the current study suggests the importance of BSSD traits in the evolution of SSD. Unlike terrestrial plethodontid salamanders (Maerz et al. 2006), in many migratory amphibians, clarification of BSSD traits that alternate between aquatic and terrestrial phases of the life cycle is a considerable contribution to our understanding of the evolution of complex life cycles (Malmgren and Thollesson 1999; Pough et al. 2001; Salvidio and Bruce 2006). For example, a noticeable increase in head width, resulting from the swelling of the whole body of the male during the aquatic phase, is related to male–male competition and is unknown in families other than hynobiids that accomplish external fertilization (Hasumi and Iwasawa 1990; Hasumi 1994, 2001a). In addition to increased head width, male *S. keyserlingii* had a longer tail than females in size and shape (i.e., sexually dimorphic trait) and had a longer tail in the aquatic phase than in the terrestrial phase (i.e., biphasically dimorphic trait). The longer tail of the aquatic-phase male may contribute to catching the fertilizable female by winding his tail around her pelvic region, a behavior that occurs just before scramble competition for monopolizing a pair of egg sacs (M. Hasumi, personal observation). This is likely explained by size-specific tail length in salamanders, which is directly related with the amount of reproductive output (Maiorana 1976), and this is a life-history trait in sexual selection (Andersson 1994). Although I could not compare dimorphic traits between aquatic- and terrestrial-phase females because of a small number of comparable females, increased body mass alone caused by egg sac formation is characteristic of the female hynobiid during the aquatic phase (i.e., other traits are constant: Hasumi 1996).

Thus, I hypothesize that ontogeny of SSD requires the development of secondary sexual characteristics in adult males of migratory salamanders with a complex life cycle, as a reproductive form of BSSD (i.e., aquatic-phase morph). This form of BSSD suggests that female-larger SSD in *S. keyserlingii* develops to resolve 'intersexual ontogenetic conflict' (Badyaev 2002) by allowing small-sized males to swell their whole body during the aquatic phase, as much as large-sized females. By contrast, in ambystomatids distributed widely in North America (i.e., a counterpart of hynobiids distributed in Asia), sexual selection on body size is lacking (Williams and DeWoody 2009). This may lead to the absence of substantial attention to the interaction of size dimorphism to a difference in shape even in *Hynobius* and *Triturus* species with drastically increased BSSD traits during the aquatic phase (Hasumi and Iwasawa 1990; Malmgren and Thollesson 1999). Speculating their interaction as suggested and hypothesized here will thus contribute to considering determinants of SSD.

Conclusions

Skeletochronological techniques developed in recent decades have been revolutionary for detecting age structure of reclusive amphibians and reptiles (Halliday and Verrell 1988). When considering independence of data, skeletochronology without using recapture data may be a unique method for analyzing age–size interaction, although some methods are presented to estimate reaction norms for age and body size at maturity (Barot et al. 2004; Griffiths and Brook 2005). While skeletochronology is a helpful tool for describing age structure of a population, application of deficient data on 0-year metamorphs and either sex only to any growth equation, as documented in many skeletochronology papers (see 'Methodological Considerations'), has induced potential overestimation of regression and growth coefficients and potential underestimation of asymptotic maximum size. Such growth trajectory problems may be common not only to migratory amphibians but also to many other animal species, for which it is not easy to capture adult individuals out of the breeding season (i.e., lack of data between years) and multi-year juveniles throughout the year (i.e., lack of data prior to maturity). Overcoming these problems with data on monthly captured and variously aged individuals will lead to a careful description and true modeling of a growth trajectory of organisms. It will then contribute to filling a gap of ontogenetic SSD requirements among vertebrate taxa such as fishes, amphibians, reptiles, birds, and mammals (Badyaev 2002; Cox and John-Alder 2007; John-Alder et al. 2007) and further to our understanding of how size dimorphism develops in indeterminate growing vertebrates. In this context, the current study supports the concept of growing apart, an ontogenetic perspective on the evolution of SSD (Badyaev 2002), and provides useful insights into the development or function of SSD as a general phenomenon in evolutionary biology.

Appendix 1

See Table 4.

Table 4 Mean measurements ± SD (range) of body mass (BM), head width (HW), tail height (TH), snout–anterior vent length (SAVL), snout–posterior vent length (SPVL), and tail length (TL) in adult males (M), aquatic-phase males (Ma), terrestrial-phase males (Mt), adult females (F), aquatic-phase female (Fa), terrestrial-phase females (Ft), unsexed individuals (U), juveniles (J), and metamorphs (N)

Class	n	BM (g)	HW (mm)	TH (mm)	SAVL (mm)	SPVL (mm)	TL (mm)
M	276	4.65 ± 1.10 (2.50–8.35)	9.43 ± 0.96 (7.46–13.76)	7.34 ± 1.05 (5.47–11.95)	52.98 ± 4.35 (41.67–64.96)	57.63 ± 4.63 (46.21–71.52)	47.72 ± 5.35 (32.04–65.45)
Ma	29	5.67 ± 1.00 (2.50–8.35)	11.45 ± 0.88 (9.73–13.76)	9.57 ± 0.95 (7.49–11.95)	56.39 ± 4.06 (42.20–64.18)	61.95 ± 4.36 (47.27–71.52)	54.85 ± 5.74 (38.04–65.45)
Mt	247	4.52 ± 1.04 (2.50–7.60)	9.20 ± 0.65 (7.46–11.01)	7.08 ± 0.70 (5.47–9.24)	52.58 ± 4.21 (41.67–64.96)	57.12 ± 4.40 (46.21–69.31)	46.89 ± 4.64 (32.04–58.32)
F	130	5.44 ± 1.12 (3.20–8.25)	9.39 ± 0.56 (8.27–10.85)	7.18 ± 0.74 (5.40–9.11)	57.52 ± 4.48 (47.24–68.72)	61.70 ± 4.56 (52.00–72.33)	43.24 ± 3.92 (34.50–52.08)
Fa	1	(6.20)	(10.30)	(8.22)	(61.04)	(65.60)	(47.60)
Ft	129	5.43 ± 1.13 (3.20–8.25)	9.38 ± 0.55 (8.27–10.85)	7.17 ± 0.74 (5.40–9.11)	57.49 ± 4.49 (47.24–68.72)	61.67 ± 4.57 (52.00–72.33)	43.21 ± 3.92 (34.50–52.08)
U	23	3.87 ± 0.71 (2.95–5.75)	8.87 ± 0.64 (7.76–10.59)	6.55 ± 0.55 (5.87–7.89)	51.10 ± 3.37 (47.81–61.06)	55.30 ± 3.71 (52.24–66.67)	41.11 ± 4.63 (30.86–48.57)
J	63	2.57 ± 0.56 (1.05–3.60)	8.10 ± 0.57 (6.50–9.66)	5.73 ± 0.58 (3.74–6.69)	42.64 ± 3.44 (34.18–47.75)	46.37 ± 3.64 (37.43–51.58)	34.53 ± 4.78 (22.32–42.98)
N	63	0.88 ± 0.24 (0.45–1.65)	6.18 ± 0.43 (5.14–7.54)	4.15 ± 0.47 (2.91–5.26)	29.10 ± 2.88 (21.88–37.44)	31.18 ± 3.05 (23.11–39.77)	21.88 ± 2.71 (13.93–28.67)

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