

# Maternal Characteristics Affect Clutch and Hatchling Dimensions in the Habu, *Trimeresurus flavoviridis*

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## ハブの母親の形質が卵と孵化個体の測定値に及ぼす影響

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**Abstract :** I measured 280 fertile female habu (*Trimeresurus flavoviridis*) and their clutches and hatchlings collected in subtropical Okinawa Island. There was considerable inter- and intra-clutch variation in the size and shape of eggs and hatchlings. The oviductal eggs were aligned between the gallbladder and vent. In clutches produced by large females and by females with high body mass condition, clutch size, clutch mass, the means of egg width and body mass condition of hatchlings were large and the mean egg length was small. In clutches produced by large females, the means of egg mass and hatchling size were large, and relative clutch mass was low. The length and width of eggs were negatively correlated to each other among clutches and in each clutch, and females which lost body mass before oviposition tended to produce unfertilized eggs without decreasing the sizes of fertilized eggs. These features indicate a tendency for females to produce eggs of a constant size. Larger clutches tended to contain small fertilized eggs. Intra-clutch variations of length, width and mass of eggs were high in females with high body mass condition. Within a clutch, a longer egg produced a relatively lighter hatchling, and a longer and heavier egg produced a hatchling of larger length and mass and of a higher mass condition. The linear alignment of oviductal eggs in the narrow abdominal cavity restricts the clutch size and reproductive investment in large female habu, and this constraint and clutch size affect the size and shape of not only eggs but also of hatchlings.

**Key words :** Egg and hatchling; Habu; Mother snake; Size and shape; *Trimeresurus flavoviridis*

### I Introduction

In snake species females continue to grow after maturation and there is considerable intra-specific size variation in the size of mature females. However, the offspring size does not increase at the same rate as maternal size. Models predict the existence of an optimal offspring size, decided, for example, by the energy expended per offspring, the fitness of the offspring, and parental fitness<sup>1)</sup>. Offspring size is determined by the amount of materials expended for the brood and the number of offspring. In most snakes the relative clutch mass is stable for a given species<sup>2,3,4)</sup> and the offspring number increases with the female size. Accordingly, in several snake species offspring size is independent of the length and mass of females<sup>5)</sup>. However, few studies on snake reproduction have considered the intra-specific variation within large

sample sizes and few studies<sup>6,7,8)</sup> have examined the intra-brood variation of offspring sizes.

In the habu, *Trimeresurus flavoviridis* (Hallowell), a viperid snake, inhabiting the subtropical Ryukyu Archipelago, Japan, the secondary vitellogenesis occurs in November and December and ovulation occurs in June<sup>9)</sup>. Habu copulate between March and May<sup>10,11)</sup>, and about half of the females<sup>12)</sup> larger than ca. 90 cm in snout-vent length<sup>13)</sup> lay eggs in July<sup>14)</sup>. The body mass of gravid females overlaps that of non-gravid ones and does not change during the pre-ovipositional several months<sup>15)</sup>. To produce a clutch, gravid females lose about 30% of their body mass, including most of the fat body mass, and larger and fatter females maintain better condition of these masses after oviposition<sup>16)</sup>. Most of the pre-ovipositional females have eggs situated linearly in the abdominal cavity, with

those in the right oviduct anterior to those in the left oviduct<sup>17,18</sup>.

Egg shape is highly variable in the habu<sup>18,19</sup>. This large variation is partly due to the fact that smaller females produce more-slender eggs<sup>20,21</sup>, indicating that egg shape is influenced by maternal size. In this paper I describe the clutch size and inter- and intra-clutch variations in the size and shape of eggs and hatchlings in reference to the length and body mass of female habu.

## II Materials and Methods

The study subjects were 280 fertile habu females collected in Okinawa Island from 1981 to 1999. After the measurement of snout-vent length (SVL) and body mass (BM) the females were maintained in captivity with food provided twice a month and water ad libitum. BM of the females was measured after oviposition or death, and the length, width and mass of oviposited or oviductal eggs were measured. As BM of the females changed in captivity, only females that oviposited within a month after collection were regarded as representing snakes of natural body condition. For the other females the difference of BM (DBM) at the time of

capture to that (including clutch) at time of oviposition was calculated.

Oviposited eggs were measured within 1.5 days (mostly 0.5 day) of being laid. Egg width was measured, assuming the section as a circle. The eggs were categorized as abnormal or normal, the former being irregularly shaped with a yellowish shell (Fig. 1). Dissection of a selection of eggs revealed that no abnormal eggs contained embryos, whereas all eggs with an embryo or with a hatchling were normal ones. In the regression analyses of egg measurements only data of oviposited normal eggs were used. The normal eggs were kept individually and SVL and BM of hatchlings were measured. Some hatchlings left yolk in the egg shell, and the BM of these hatchlings was excluded from the analyses using hatchling BM.

The abbreviations are summarized in Table 1. BM condition (BMC) of a female and that of a hatchling are the residuals of BM from the regression line of BM on SVL, in the females of natural body condition and in hatchlings that left no yolk in the egg shell, respectively. I used SVL, BMC and DBM to analyze the effects of female conditions on egg and hatchling formation. In

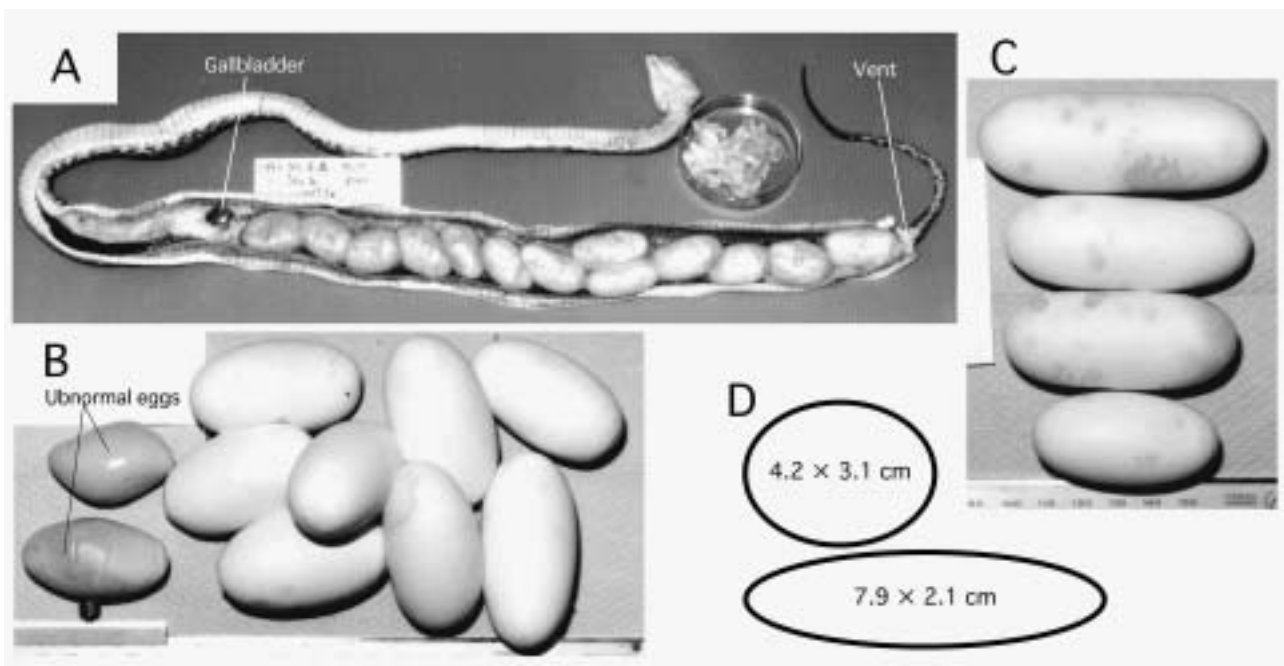


Fig. 1. A gravid female and eggs of the habu (*Trimeresurus flavoviridis*). A: Female and oviductal eggs between the gallbladder and vent; B: a clutch with abnormal eggs; C: a clutch with large variation in egg length; D: schematic shape of most slender and most round eggs.

Table 1. Abbreviations of the variables referred to in this paper.

Abbreviation	Definition
SVL	Snout-vent length (cm)
BM	Body mass (g)
BMC	BM condition (residual from the standard BM)
DBM	Change in BM from the time of capture to oviposition (g)
DGV	Distance from gallbladder to vent (cm)
RCM	Relative clutch mass: [clutch mass] / [post-ovipositional BM]
RHE	Ratio of [hatchling mass] / [egg mass]

several analyses I categorized females into one of two to six groups, assuming similar group sizes under the conditions of normal distribution. In the case of categorizing female BMC, SD of BMC in females of natural condition was utilized. When grouping females by DBM, q was configured as 0.434 of SD of BMC (1/6 of BMC is expected to be between 0 to q). For the regression analyses and the statistical tests the measurements were transformed to natural logarithms.

### III Results

#### 1. Size and feeding of females

SVL of the females ranged from 87.0 to 159.5 cm (Table 2). The regression equation of BM on SVL in females of natural body condition was;

$$\text{Log BM} = 3.28 \text{ Log SVL} - 9.33, N = 65,$$

$$R^2 = 0.897, P < 0.001$$

Among 39 gravid females fed in June and July and 38 post-ovipositional females provided with mice in July and August, 9 and 15 ate, respectively. Among these gravid and post-ovipositional females that fed the shortest three periods before and after the oviposition were 10, 18 and 38 days, and 1, 2 and 3 days, respectively. These results show that pre-ovipositional females fed less frequently than do post-ovipositional females.

#### 2. Egg arrangement in the oviducts and egg sizes

Some pre-ovipositional females had oviductal eggs, which occupied almost the whole abdominal cavity (Fig.1). The mean distances from gallbladder

to oviductal eggs and from oviductal eggs to vent in the pre-ovipositional females which died between 25 June and 25 July (within the ovipositing period) were 1.52 cm (N = 27, SD = 2.25, range -1.6 to 7.5) and 5.43 cm (N = 27, SD = 2.28, range 0.5 to 10.0), respectively. The mean proportion of DGV (distance from gallbladder to vent) to SVL was 0.337 (N = 144, SD = 0.0204). The slope of the regression line of DGV on SVL (both in logarithmic values) was 1.117, a value significantly larger than 1 (N = 144, P < 0.001). Sum of all egg lengths per DGV was positively correlated with SVL (N = 134, R<sup>2</sup> = 0.159, P < 0.001, Fig. 2). The ratio is greater than one in the females of SVL larger than about 4.7 in logarithm (110 cm). In large females, eggs were slanted in the oviducts. Five females (112.0, 119.5, 125.0, 138.0 and 149.0 cm in SVL) were detected to have oviductal eggs lined side by side (Fig. 1). In small females the oviductal eggs were oriented with their long axis parallel to the body axis, and sum of all egg lengths were close to or less than DGV.

The clutch sizes and egg dimensions are shown in Table 2. The minimum mass of egg with hatchling was 17.2 g. The normal oviposited eggs of mass less than 17.2 g (N = 11) were larger than abnormal oviposited eggs (N = 88) in mass (P < 0.01, Mann-Whitney's test). In the following analyses on egg sizes, unless stated otherwise, eggs are oviposited normal ones of mass equal to or greater than 17.2 g, and clutches are those with the mean egg mass equal to or greater than 17.2 g.

There was considerable inter- and intra-clutch variation in the size and shape of eggs. The mean egg mass in a clutch was correlated with the means of both egg length and width, and the latter two were negatively correlated with each other (Table 3).

The deviation of egg width from the mean of each clutch was negatively correlated with that of egg length (N = 319, R<sup>2</sup> = 0.185, P < 0.001). The deviation of egg mass from the mean of each clutch was positively correlated with that of egg length (N = 317, R<sup>2</sup> = 0.513, P < 0.001, Fig. 1, 4)

Table 2. Female SVL, number of eggs per clutch and measurements of oviposited eggs in the habu (*Trimeresurus flavoviridis*).

	N	Mean	SD	Min	Max
Female SVL (cm)	279	117.2	12.9	87.0	159.5
Clutch size	276	7.03	2.47	2	15
Number of normal eggs	240	6.54	2.94	0	13
Number of abnormal eggs	194	0.74	1.69	0	10
Egg length (cm)					
Hatched	370	5.55	0.70	4.25	8.30
Normal, $\geq 17.2$ g	977	5.57	0.76	4.00	8.75
Normal, $< 17.2$ g	11	4.61	0.64	3.55	6.00
Abnormal	74	4.40	0.67	3.45	6.95
Egg width (cm)					
Hatched	343	2.87	0.21	2.10	3.60
Normal, $\geq 17.2$ g	940	2.87	0.23	2.10	3.60
Normal, $< 17.2$ g	11	2.30	0.21	2.05	2.80
Abnormal	54	2.05	0.23	1.15	2.50
Egg mass (g)					
Hatched	356	27.1	4.9	17.2	43.9
Normal, $\geq 17.2$ g	994	27.1	5.2	17.2	45.3
Normal, $< 17.2$ g	11	14.4	2.1	11.0	17.1
Abnormal	83	10.5	2.2	3.5	16.2

and that of egg width ( $N = 296$ ,  $R^2 = 0.016$ ,  $P < 0.05$ ). The coefficient of variance (CV) of length, width and mass of eggs (Table 2) were 0.136, 0.080 and 0.192, respectively, and respective means of these CV of each clutch were 0.075 ( $N = 138$ ), 0.033 ( $N = 128$ ) and 0.080 ( $N = 127$ ). CV of egg length in each clutch was larger than that of egg width ( $P < 0.001$ , Wilcoxon signed ranks test). CV of egg length and CV of egg width were positively correlated with each other and with CV of egg mass (Table 3).

### 3. Effects of female SVL on the clutch

Regression analyses of each measurement on each and several variables were done for clutches with oviposited eggs (Table 3, 4). Among the three variables on female conditions, DBM was indicated as being significantly important in none of the analyses. In contrast, both SVL and BMC were significantly important factors in all the multiple

Table 3. Simple regressions between the investigated variables in the habu. Ps are  $>0.05$  (ns),  $<0.05$  (\*),  $<0.01$  (\*\*), and  $<0.001$  (\*\*\*), and those with "-" are negatively correlated. <sup>1)</sup>: each measurement or mean (with thin underline) or CV (with thick underline) or deviation (with double underline) in the clutch.

	Female SVL		Female BMC		RCM		Clutch size		Egg length <sup>1)</sup>		Egg width <sup>1)</sup>		Egg mass <sup>1)</sup>	
	N	Slope, P	N	P	N	P	N	P	N	P	N	P	N	P
RCM	174	-0.210*	174	***			175	***						
Clutch size	182	2.09***	174	***	175	***								
Clutch mass	174	2.92***	174	***	175	***	175	***						
Sum of all egg lengths	151	1.96***	149	***	149	***	151	***						
Mean egg length	163	-0.190*	160	-*	160	-***	163	-***						
Mean egg width	154	0.544***	153	***	153	ns	154	***	154	-**				
Mean egg mass	169	0.569***	168	ns	169	-***	170	ns	155	***	154	***		
(Mean) SVL of hatchlings	101	0.155**	99	ns	100	-**	102	ns	359	***	332	***	345	***
(Mean) BM of hatchlings	54	0.940***	52	*	53	-*	55	ns	163	***	141	**	175	***
(Mean) BMC of hatchlings	53	0.600***	51	*	52	ns	54	*	154	***	132	***	166	***
(Mean) RHE	51	ns	51	ns	52	ns	52	ns	160	-**	141	ns	175	ns
CV of egg length	128	ns	126	*	126	*	127	**						
CV of egg width	123	ns	121	**	121	**	122	**	123	***				
CV of egg mass	120	ns	118	*	119	ns	120	ns	114	***	109	**		
Deviation of egg width									319	-***				
Deviation of egg mass									317	***	296	*		
Deviation of hatchling SVL									335	***	308	ns	306	***
Deviation of hatchling BM									144	***	126	ns	144	***
Deviation of hatchling BMC									136	*	118	ns	136	***
Deviation of RHE									144	-*	126	ns	144	ns

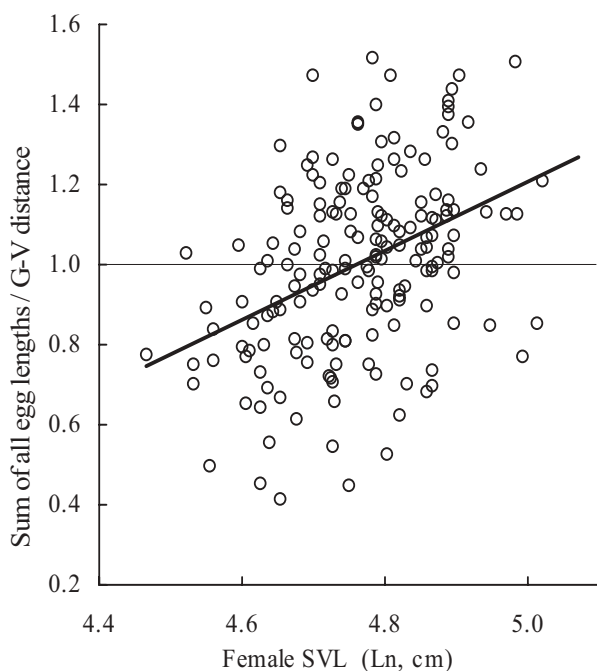


Fig. 2. Female SVL and [sum of lengths of all oviposited eggs] / [DGV: distance from the gallbladder to vent] in the habu.

regression analyses of the variables on clutch and egg (Table 4). I analyzed the effects of SVL on clutch formation firstly, then those of BMC, and compared the residuals of other variables from the multiple regression line on SVL and BMC.

The changes in the variables with the changes in SVL at standard BM (BMC = 0) are shown in Fig. 3. Clutch size, clutch mass, the means of width and mass of eggs were positively correlated with female SVL, whereas RCM and the mean egg length were negatively correlated with it (Table 3,

4, Fig. 3).

The negative correlation of RCM was due to a slope of clutch mass smaller than that of BM. I compared the slopes of regression lines of several variables on SVL. The slope of regression lines on SVL were 1.117 for DGV and  $-0.229$  for the mean egg length, which facts allow the clutch size to increase with SVL by  $1.117 - (-0.229) = 1.346$ . The observed slope, 2.215, was the result of the overlapping of the long axis of eggs by slanting the axis and by double-lining the eggs within the abdominal cavity. The mean egg mass increased with SVL by a slope of 0.710, which allows the slope of clutch mass,  $2.215 + 0.710 = 2.925$ , close to the observed 3.09. However, this slope is still less than that of BM, 3.28, which ultimately results in a decrease of RCM with an increase in SVL.

#### 4. Effects of female BMC on the clutch

The changes in the variables with the changes in BMC at the mean SVL changes are shown in Fig. 3. Clutch size, clutch mass, the mean egg width and RCM were positively correlated with female BMC, whereas the mean egg length was negatively correlated with it (Table 3, 4, Fig. 3). Among the female conditions, only BMC was positively correlated with CV of each measurement of eggs in a clutch (Table 3). Among other variables clutch size and RCM were positively correlated with both CV of egg length and that of egg width.

Table 4. Multiple regressions of the investigated variables in the habu. Ps are  $>0.05$  (ns),  $<0.05$  (\*),  $<0.01$  (\*\*) and  $<0.001$  (\*\*\*).

	N	Intercept	Female SVL		Female BMC		P	R <sup>2</sup>
			Slope	F	Slope	F		
RCM	174	1.325	-0.173	32.7	0.326	26.0	***	0.163
Clutch size	174	-8.636	2.215	167.2	1.104	60.2	***	0.551
Clutch mass	174	-9.569	3.094	712.9	1.495	241.6	***	0.838
Total length of eggs	149	-6.180	2.061	293.0	1.014	98.7	***	0.712
Mean egg length	160	2.835	-0.229	6.6	-0.183	6.3	**	0.067
Mean egg width	153	-1.815	0.596	578.9	0.280	189.6	***	0.821
Mean egg mass	168	-0.102	0.710	42.6	0.263	8.2	***	0.219
SVL of hatchlings	99	2.690	0.169	10.8	ns		**	0.100
BM of hatchlings	52	-2.253	1.079	33.8	0.624	15.0	***	0.457
BMC of hatchlings	51	-3.149	0.664	29.6	0.433	16.7	***	0.427
RHE	51						ns	

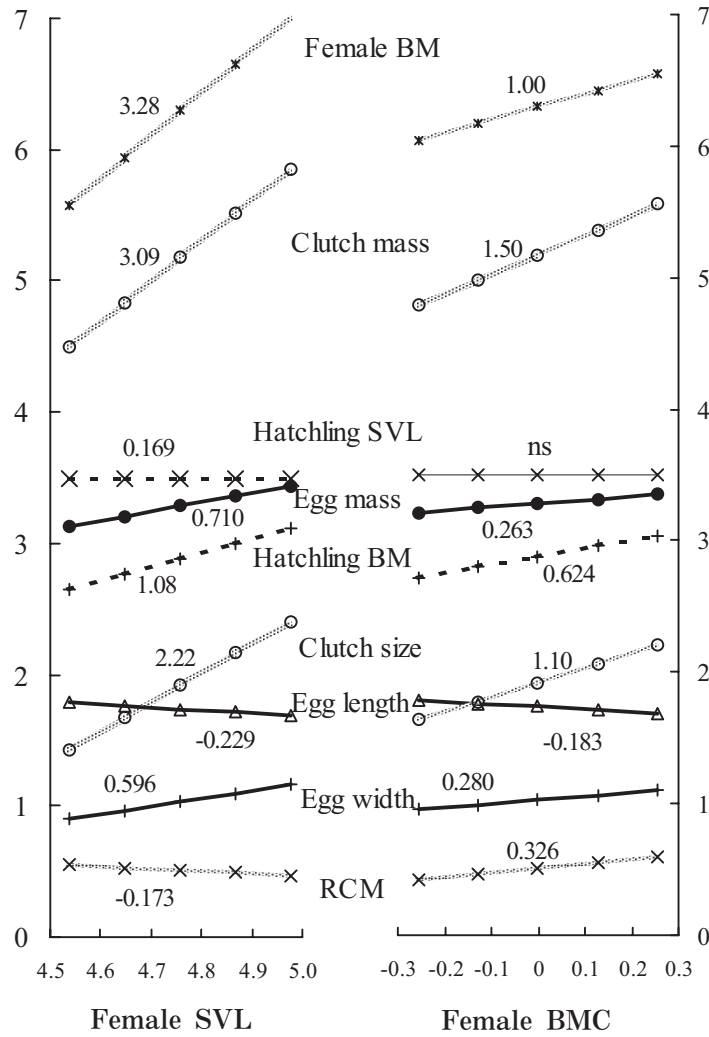


Fig. 3. The changes in the variables with the changes in female SVL at standard BM (BMC = 0) and those in female BMC at the mean SVL in the habu. The symbols are plotted with the interval of SD of SVL and that of BMC. The slopes of the variables are the partial regression coefficients on SVL and BMC.

Among the minimums and maximums of length, width and mass of eggs, significant correlations with BMC were detected in the minimums of egg length (negatively,  $N = 159$ ,  $P < 0.01$ ), the minimums and maximums of egg width (both positively,  $N = 154$ ,  $P < 0.001$ ) and the maximums of egg mass (positively,  $N = 151$ ,  $P < 0.01$ ). A high BMC increased egg width and decreased length of several eggs, which in turn gave rise to the large masses of several eggs in each clutch.

5. Residuals from the multiple regression line on SVL and BMC

The residuals of RCM from the multiple regression line on SVL and BMC ( $N = 150$ ,  $R^2 = 0.474$ ,  $P < 0.001$ ) were positively correlated with the

residuals of clutch size, whereas those of mean egg length ( $N = 144$ ,  $R^2 = 0.621$ ,  $P < 0.001$ ) and of mean egg mass ( $N = 149$ ,  $R^2 = 0.580$ ,  $P < 0.001$ ) showed a negative correlation. The residuals of mean egg mass were negatively correlated with those of RCM ( $N = 148$ ,  $R^2 = 0.089$ ,  $P < 0.001$ ), which means that in females with high RCM clutch size increased greater than clutch mass. The residuals of mean egg width were not correlated with those of mean egg length ( $N = 138$ ,  $P > 0.05$ ), so the negative correlation between these two means was attributed to the effects of SVL and BMC on these means.

6. DBM and abnormal eggs

The proportions of clutches with abnormal eggs



Table 5. BMC and DBM of habu females and the frequencies of clutches with abnormal eggs.  $q: 0.434 \times SD$  of BMC (1/6 of BMC is expected to be between 0 to  $q$ ).

	BMC < -q			-q < BMC < q			q < BMC			Total		
	N	With abnormal		N	With abnormal		N	With abnormal		N	With abnormal	
		N	%		N	%		N	%		N	%
Females oviposited within one month after capture	15	4	26.7	24	1	4.2	25	1	4.0	64	6	9.4
Females kept more than a month before oviposition												
DBM < -q	24	12	50.0	9	4	44.4	8	5	62.5	41	21	51.2
-q < DBM < 0	13	2	15.4	15	5	33.3	9	3	33.3	37	10	27.0
0 < DBM < q	14	2	14.3	10	0	0.0	13	1	7.7	37	3	8.1
DBM > q	1	0	0.0	5	0	0.0	5	2	40.0	11	2	18.2
Total	52	16	30.8	39	9	23.1	35	11	31.4	126	36	28.6

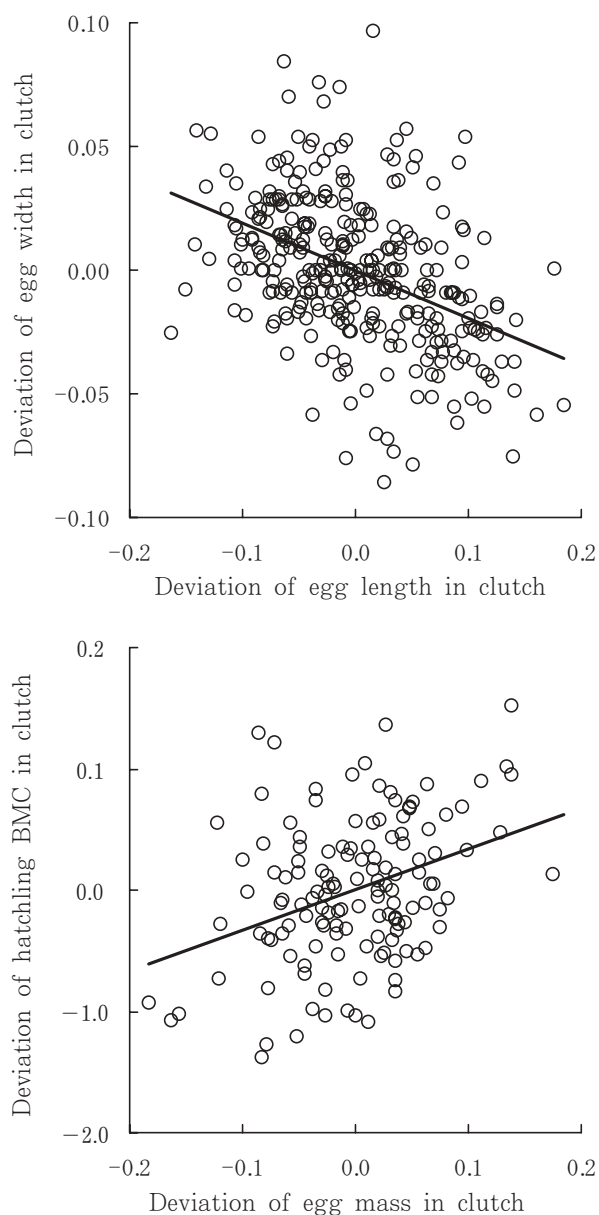


Fig. 4. Deviations of egg length and those of egg width from the mean of each clutch (upper) and those of egg mass and those of hatching BMC in the habu.

laid by females collected from March to May (14/32) and those collected in June (17/42) were not significantly different ( $P > 0.05$ , Fisher's exact test). Between clutches with abnormal eggs ( $N = 53$ ) and those with only normal eggs ( $N = 181$ ), DBM and RCM were significantly different (both  $P < 0.001$ , Mann-Whitney's test), being smaller and higher in the females with abnormal eggs, respectively.

To compare the effects of female DBM and BMC on the production of abnormal eggs, females at oviposition were also categorized to four groups of DBM: less than  $-q$ ,  $-q$  to 0, 0 to  $q$ , and greater than  $q$ , and three groups by BMC: less than  $q$ ,  $-q$  to  $q$ , and greater than  $q$ . The frequency of abnormal eggs was high in females of small DBM, and was not high in females of low BMC (Table 5). By dividing the females into four SVL classes, it was evident that the largest female group produced fewer clutches (7/57 vs. 46/176 in the other groups) with abnormal eggs ( $P < 0.05$ , Fisher's exact test).

Between clutches with or without abnormal eggs in females with negative DBM, the residuals of RCM (respective  $N = 30, 18$ , as also for in the following data,  $P < 0.05$ , t-test) and of number of normal eggs ( $N = 46, 20$ ,  $P < 0.001$ , Mann-Whitney's test) were larger in these clutches without abnormal eggs, whereas the residuals of clutch size ( $N = 46, 20$ ) and of means of egg length ( $N = 29, 17$ ), width ( $N = 27, 15$ ) and mass ( $N = 30, 18$ ) were not significantly different between clutches with or without abnormal eggs (all  $P > 0.05$ , t-test).

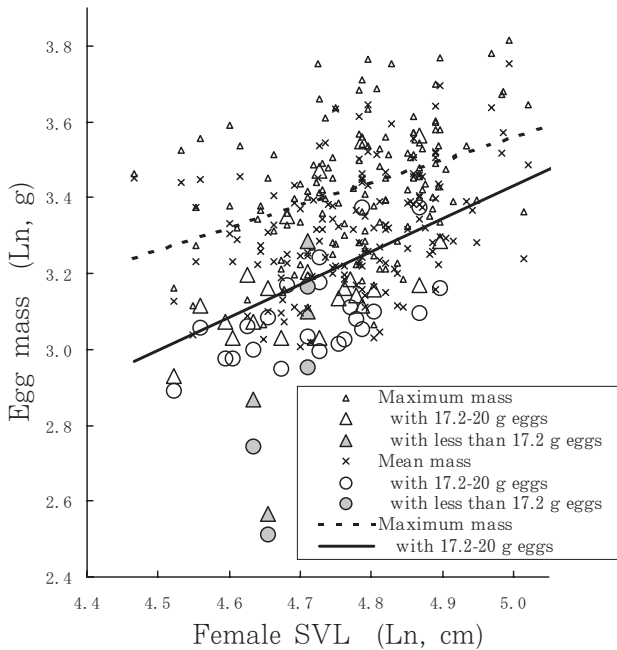


Fig. 5. Female SVL and the mean and maximum egg masses of the clutches with oviposited eggs (with and without small eggs; small eggs are grouped into two groups) in the habu.

### 7. Small eggs

The means of egg mass were less than 17.2 g (the lowest mass of an egg with hatchling) in three clutches. The BMC of these three clutches was in the lowest class (N = 19) among the six groups of BMC (N of the other groups = 132, 3/19 vs. 0/132,  $P < 0.01$  Fisher's exact test), and both the two females kept more than one month were in the lowest class among the four DBM groups.

To assess the effects of forming small eggs, clutches with small normal eggs (provisionally less than 20 g, N = 27) were compared with those containing all normal eggs of more than 20 g (N = 157). The clutches with small normal eggs showed small means of egg mass and small maximum egg mass (both  $P < 0.001$ , Mann-Whitney's test, Fig. 5). Therefore, in these clutches, most or all eggs were small. Clutches with small eggs were fewer in the female group with larger SVL (7/94) than in that with smaller SVL (20/89) ( $P < 0.001$ , Fisher's exact test, Fig. 5). The residuals of the clutch size from the multiple regression line on SVL and BMC were large in the females with egg mass less than 20 g (N = 27,  $P < 0.001$ , Mann-Whitney's test), while

those of RCM were not different between females with small eggs or those without them (N = 148) ( $P > 0.1$ , Mann-Whitney's test). Therefore, these clutches with small eggs were formed by increasing their sizes without attaining the additional mass for the eggs.

### 8. Eggs and hatchlings

Both the lengths and masses of eggs were larger in hatchlings that left yolk in the shell than in those that left no yolk (N = 47, 164 and 45, 176,  $P < 0.05$  and  $< 0.01$ , respectively, t-test). The proportion of female hatchlings that left yolk in the egg shell (35/97) was higher than in males (11/121) ( $P < 0.001$ , Fisher's exact test). Among the females producing two or more hatchlings there were no significant differences in SVL (N = 27, 22), BMC (N = 26, 21) nor DBM (N = 26, 18) between females that produced hatchlings that left yolk in the egg shell and those that did not (all  $P > 0.1$ , t-test and Mann-Whitney's test).

The means of both SVL and BMC of the hatchlings in each clutch were correlated with female SVL, and the mean BMC of the hatchlings in each clutch was correlated with female BMC (Table 3). The mean RHE (ratio of [hatchling mass] / [egg mass]) in each clutch was not correlated with SVL, BMC, DBM or RCM of the females (Table 3).

The CV of SVL and BM of hatchlings were 0.0161 and 0.0615, respectively, and respective means of these CV of each clutch were 0.0082 (N = 84) and 0.0181 (N = 42). CV of hatchling BM in each clutch was larger than that of hatchling SVL ( $P < 0.001$ , Wilcoxon signed ranks test).

The slopes of the regression equation (Table 3) between egg mass and hatchling SVL and between egg mass and hatchling mass were 0.227 (N = 345,  $R^2 = 0.359$ , smaller than 0.333,  $P < 0.001$ , t-test) and 0.950 (N = 175,  $R^2 = 0.863$ , smaller than 1.0,  $P < 0.05$ , t-test), respectively. Therefore, heavy eggs did not produce, for their sizes, large hatchlings in length and mass. Egg mass was correlated negatively to the ratio of [hatchling SVL] / [egg



Table 6. Multiple regressions of the hatchling variables on the egg variables in the habu. Ps are >0.05 (ns), <0.05 (\*), <0.01 (\*\*), and <0.001 (\*\*\*).<sup>1)</sup>: each measurement or deviation in the clutch.

	N	Intercept	Egg length <sup>1)</sup>		Egg width <sup>1)</sup>		P	R <sup>2</sup>
			Slope	F	Slope	F		
Hatchling SVL	332	2.633	0.255	102.1	0.411	98.8	***	0.354
Hatchling BM	141	-0.428	1.017	303.4	1.554	274.4	***	0.811
Hatchling BMC	132	-1.785	0.491	51.5	0.930	74.3	***	0.503
RHE	141	0.838	-0.083	6.3	ns		*	0.044
Deviation of hatchling SVL	308	0.000	0.237	63.5	0.302	21.3	***	0.176
Deviation of hatchling BM	126	0.002	0.909	200.8	1.238	59.2	***	0.623
Deviation of hatchling BMC	118	0.000	0.323	12.1	0.573	6.7	**	0.104
Deviation of RHE	126	0.000	-0.062	5.3	ns		*	0.041

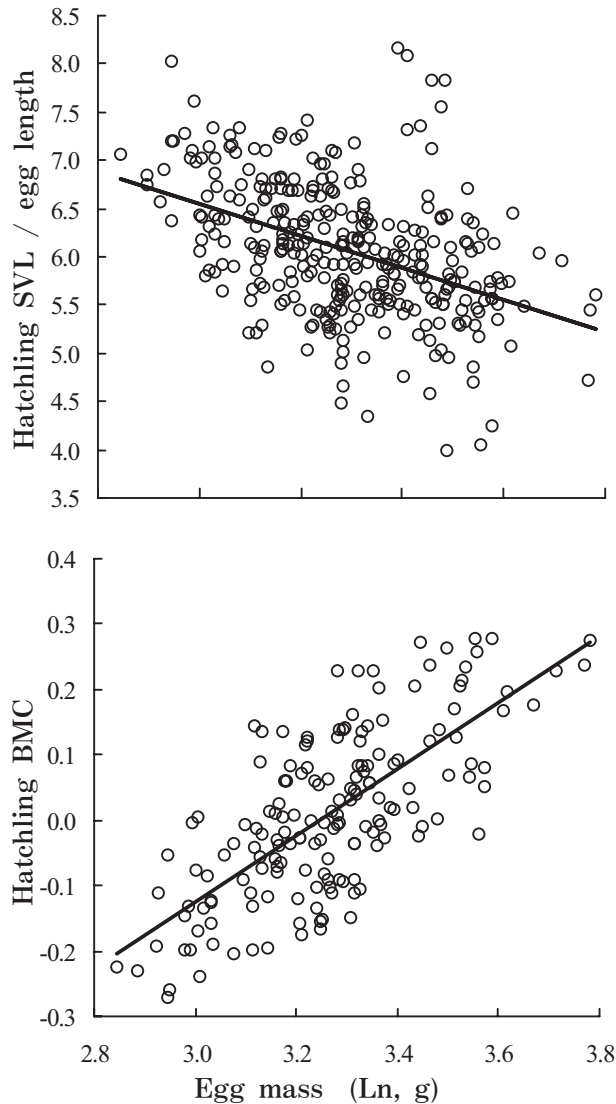


Fig. 6. Relationships of egg mass to hatchling BMC and [hatchling SVL] / [egg length] in the habu.

length] (N = 326, R<sup>2</sup> = 0.185, P < 0.001, Fig. 6). In small eggs the body of embryo just before hatching was folded more than six times. Hatchling BMC was positively correlated with length, width and mass (Fig. 6) of eggs (Table 3). RHE was correlated significantly only with egg length negatively.

In the multiple regression analyses, SVL, BM and BMC of hatchlings were correlated positively with both length and width of eggs, while RHE was correlated negatively only with egg length (Table 6). These facts were similar in the multiple regression analyses of the deviations of the variables from the means of each clutch (Table 6).

#### IV Discussion

##### 1. Clutch size and unfertilized egg

Habu females with large SVL and high BMC produced large clutches. Many studies of snake reproduction have shown that large females produce large broods<sup>22)</sup> and several have noted that fat females have large broods, e.g. in the water snake, *Nerodia sipedon*<sup>23)</sup>, the adder, *Vipera berus*<sup>2)</sup>, and the water python, *Liasis fuscus*<sup>9)</sup>. In snakes, all mature eggs are oviposited at one time and pre-ovipositional eggs are lined up in each of the right and left oviduct. In pre-ovipositional female habu, the oviductal eggs nearly filled the abdominal cavity, and the DVG is close to the maximum

length of the egg column in the abdominal cavity. In small female habu the oviductal eggs are aligned with their long axis parallel to the body axis under the constraints of the width of the abdominal cavity, and so the sum of all egg lengths was close to the DGV. Therefore, in these females the maximum clutch size is decided by the egg length and the DGV (or SVL). In order to increase the clutch size, in theory the egg length should be decreased. However, owing to the limitations imposed on the egg width in small females this would result in a decrease of egg mass.

In large females the sum of all egg lengths exceeds the DGV, because the oviductal eggs are aligned slanting and partially overlapping. However, even in large females only one or two eggs were overlapped. In order to double the profile of the abdominal cavity to double-line the most slender eggs of the smallest mature females, the SVL should increase by at least 1.41 (square root of 2) times, which indicates an SVL of 123 cm ( $87 \times 1.41 = 123$  cm) as the minimum size. In the present study the double-lined eggs were wider than those of the most slender ones, and in middle-sized females the double-lined eggs were pointed. The double lining of oviductal eggs does not seem an easy strategy for the habu.

Some females produced clutches with abnormal eggs, which may be regarded as unfertilized and thus do not contribute to female fecundity. Females, which lost BM before oviposition, tended to produce more unfertilized eggs and fewer fertilized eggs. These females are estimated to have prepared enlarged follicles in numbers appropriate to the former BM and to stop the growth of several follicles after losing BM. However, the residuals of RCM were smaller in these clutches with unfertilized eggs. The factors which result in females with low DBM producing unfertilized eggs or having high RCM without unfertilized eggs are unknown. Large habu are fatter than smaller habu, and this ample energy reserve might help them maintain the growth of follicles even under conditions of low DBM.

Energetically it is preferable for females of low DBM to not produce unfertilized eggs but to resorb some of the enlarged follicles. In the southeastern crowned snake, *Tantilla coronata* atresias of vitellogenic follicles occur normally<sup>24</sup>. In *Crotalus viridis*, females suffering transmitter implantation in the early season resorbed their follicles<sup>25</sup>. In the lizard *Uta stansburiana*, injection of follicle-stimulating hormone increased the clutch size and produced smaller eggs, and follicles were recruited after yolkectomy at early vitellogenesis, whereas females with late yolkectomy produced small clutches with large eggs<sup>26</sup>. These studies show that in squamates, the number of enlarged follicles can be changed in the early stages of follicular growth. In the habu, secondary vitellogenesis occurs in October or November and follicles grow rapidly in April or May to a length of more than 3 cm<sup>9,12</sup>. The proportions of clutches containing abnormal eggs were similar between early and late seasons of collection. Although habu might regulate the number of enlarged follicles, the BM changes in the present samples may have occurred too late for regulation to have been effected.

The present results suggest that pre-ovipositional females feed less frequently than do post-ovipositional females. Gravid snakes are less common in June and July than in spring<sup>12</sup>, suggesting that they do not forage actively in the pre-ovipositional period. However, the standard body mass of fertile females does not change in the pre-ovipositional season in the field<sup>15,16</sup>. These characteristics reflect the low rate of weight loss seen during periods of no feeding in the habu<sup>27</sup>. However, some fertile females may lose considerable BM in the field, because in the present study several females of natural body condition produced unfertilized eggs.

## 2. Egg size and its variation

The present results show that the egg size variation in the habu was large, even among fertilized eggs, and the regression analyses show

that both SVL and BMC of females were important factors affecting the means of length, width and mass of eggs. In the habu, the volume of the abdominal cavity and other eggs may restrict the growth of oviductal eggs<sup>20,21)</sup> and cause shape modification of some eggs within a clutch<sup>18)</sup>. The means of egg length and width were negatively correlated with each other, resulting in egg mass tending to become constant. This tendency was also observed in each clutch; an egg with its length larger than the mean of the clutch has a smaller than average width.

The mean egg (normal) masses were not different between the clutches with unfertilized eggs and those without them, indicating that females of low DBM maintain the size of fertilized eggs similar. In viviparous *Thamnophis marcianus* there were no differences of offspring size between females of high and low energy input<sup>7)</sup>.

Although three clutches with fertilized eggs of the smallest size group were produced by females with low BMC and DBM, there were no notable features in BMC and DBM in females which produced clutches with small eggs (< 20 g). The smaller eggs tended to occur in clutches of greater than average size, while RCM was not different between females with or without small eggs. Therefore, these females might have formed too many enlarged follicles initially, and subsequently been unable to regulate their growth. In the viviparous snake *Virginia striatula*, removal of a single egg or the entire eggs from one oviduct did not cause any differences in the remaining neonates between the treatments<sup>28)</sup>. The size of ovulated egg may not change by the reduction of their number, even in viviparous species.

In the female groups of low DBM, with the appearance of smaller-sized unfertilized eggs, the egg width was larger. The females with low DBM may lose fat body mass and carcass mass greatly<sup>16)</sup>. These decreases may make the abdominal cavity wider and provide some extra space for egg width.

In clutches of females with high BMC, the mean egg width and RCM were large and the mean egg

length was small. A high RCM produced a large clutch with short and light eggs. Therefore, in females with high BMC, egg mass was seldom large. In the females with high BMC not only the egg width, but fat body mass and carcass mass are also large<sup>16)</sup>. Therefore, these females have eggs packed in a narrow abdominal cavity with considerably fat body.

In several snake species larger females produce larger eggs or neonates, e.g. *V. berus*<sup>29)</sup>, *Opheodrys aestivus*<sup>30)</sup>, *Elaphe rufodorsata*<sup>31)</sup>, as do larger and heavier (high BMC) females, e.g. *T. butleri*<sup>32)</sup>, *Nerodia rhombifer*<sup>33)</sup>. In the adder, *V. berus*, abundant prey and high weight status resulted in large neonate size<sup>2)</sup>. In other taxa, however, the effects of a tradeoff between the size and number of neonates and eggs obfuscate the positive effects of maternal size and condition, e.g. *N. sipedon*<sup>23)</sup>, *L. fuscus*<sup>8)</sup> and *Storeria dekay*<sup>34)</sup>.

Several studies of snakes, including those on the habu<sup>16,20,21)</sup> have detected intra-specific variations of egg shape, but few studies of snake reproduction have considered the size variation of offspring within a brood. Egg length is more variable than egg width in several snake species, and the fact is attributed to the packing of eggs in the linear oviduct of fixed length<sup>6)</sup>. In offspring of viviparous snakes BM is more variable than SVL<sup>6)</sup>. These tendencies were documented also in the present results.

BMC was positively correlated with the CV of each measurement of eggs in the habu. Females of high BMC produced wide eggs of large numbers with several eggs of short length. Similarly, variance in neonate SVL was higher in females receiving more food in viviparous snake, *T. marcianus*<sup>7)</sup>. The mechanisms and functions of these variations in matter allocation to neonates are unknown.

### 3. Relative clutch mass

Few studies<sup>35)</sup> on reproduction in snakes and lizards have detected correlations between relative clutch mass (RCM, thereafter including CM/BM) and body size, e.g. no correlations of relative clutch

mass with SVL (in many species<sup>3,4</sup>), with BM (in *V. aspis*<sup>5</sup>), nor with yearly mean of BMC (in *V. berus*<sup>2</sup>). In the habu, RCM was correlated positively with BMC<sup>16</sup>. In the adder, *V. berus* a high prey and high weight status resulted in a small variation of RCM, and a low prey and low weight status resulted in a high variation of RCM without correlating with SVL<sup>2</sup>.

In the habu, RCM was correlated negatively with SVL. If the egg size is constant and the eggs align straightly in the abdominal cavity, the clutch size of habu increases linearly with SVL. The realized clutch size increase more rapidly by the overlapping of the long axis of eggs by slanting the axis and by overlapping the eggs within the abdominal cavity. Even with that result and the increase of mean egg mass with SVL, clutch mass increases less not so much as BM, which ultimately results in a decrease of RCM with an increase in SVL. Large females cannot produce many eggs for their BM, but keep their post-ovipositional BMC high<sup>16</sup>. Detailed studies with excluding the effects of BMC, may detect this trend between SVL and RCM to be present also in other snake species.

Smaller females produce small and slender eggs with high RCM. These eggs will produce small hatchlings of low BMC. The higher RCM results in a low BMC of females after oviposition. However, these small females produce their offspring by avoiding the probability of death (0.3/yr<sup>15</sup>) until the next season.

In some snake species, females acquiring sufficient BMC are supposed to breed<sup>22</sup>, but in the habu BMC of breeding and non-breeding females overlapped each other in more than 80% of adult females, the relative mass of both clutch and fat body fluctuated more than BMC, and females with high BMC showed high RCM<sup>16</sup>. Consequently, in the habu BMC dictates clutch mass, rather than the possibility of breeding. The present results indicate that the decrease in BMC of fertile females also causes the decrease in clutch mass by forming small unfertilized eggs.

#### 4. Eggs and hatchlings

Some hatchlings left yolk in the egg shell. Previous analysis showed the frequency of such hatchlings to be low in the wide eggs produced by the large females<sup>19</sup>. However, in the present analyses with a larger sample size, both the length and mass of eggs of hatchlings which left yolk in the shell were larger than of hatchlings which left no yolk. The mechanisms whereby more female hatchlings left yolk in the egg shell than did male hatchlings is unknown.

Among the hatchlings with completely absorbed yolk, those from slender eggs were of poor conditions in BM and in residual yolk mass<sup>19</sup>. However, the present results showed that both length and width of egg were positively correlated to hatchling BMC.

The hatchlings produced from heavy eggs did not have large SVL and BM, considering their egg mass, but high BMC, i.e. the production of larger eggs is not so efficient. Longer eggs were less efficient in hatchling mass. In the habu one or two oviductal eggs left several days in the oviduct had larger lengths<sup>18</sup>. These eggs may become longer in the nearly empty oviduct by absorbing water. Some of the longer eggs in the present sample might contain additional water, and produce light hatchlings for their mass. A female of large SVL produced hatchlings of large SVL and high BMC, and a female of higher BMC produced hatchlings of high BMC. If hatchlings of larger SVL are more advantage, larger females produce longer hatchlings of lower BMC. Among the fullterm embryos which died in the eggs, several were terata of ball-shaped conglutinate body, and others were of normal shape with making slits on the egg shell for hatchling. A long embryo may have higher risk of these deaths by folding its body more times in the shell.

The size and condition of body of the female habu affect the number, size and shape of eggs, and larger females had larger eggs of relatively smaller numbers. Large females may produce large and fat hatchlings, regardless of the degrees of fitness of these hatchlings. These tendencies

may exist in animal species with the eggs aligned linearly in its body.

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