Female Reproductive Cycle and Embryonic Development of the Chinese Mamushi (*Agkistrodon blomhoffii brevicaudus*)†

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Abstract. *-Agkistrodon blomhoffii brevicaudus* is a species of snake with seasonal reproduction. The female annual reproductive cycle is as follows: vitellogenesis begins in late March or early April; ovulation is in middle June and parturition is in middle or late August, or in early September. The gestation period lasts for about 65-75 days. By ovoviviparity, the Chinese Mamushi produces one clutch per year. In each clutch, there are 5 to 20 juveniles with a length of 154 to 203 mm and a weight of 2.0 to 5.3 g. The size of fat bodies is inversely proportional to that of the vitellogenesis. The fat bodies were larger in March and before hibernation (about November) than in the other months. The process of embryonic development is described by the external morphological investigation on 731 embryos from 82 females.

Key words: Reptilia, Serpentes, *Agkistrodon blomhoffi brevicaudus,* Chinese Mamushi, reproduction, embryonic development.

Introduction

The Chinese Mamuashi (*Agkistrodon blomhoffii brevicaudus*) is distributed mainly over southern Liaoning, Hebei, Jiangsu, Anhui, Zhejiang, Jiangxi, northern Fujian, Taiwan, Hubei, Shaanxi (southern part of the Qinling Mountains), southeastern Gansu, Sichuan and Guizhou provinces in China. It is also scattered over the Korean peninsula. It is a species of poisonous snake with a high medical value, so it attracts many scientists' attention. This paper reports the female reproductive cycle, embryonic development and reproduction of the Chinese Mamushi. It is hoped that this paper will serve as a reference material for research in reproductive biology and the artificial breeding of snakes.

Methods

Mature female Chinese Mamushi (*A. blomhoffii brevicaudus*), 447-680 mm (mean 530 mm) in length and 31-120 g (mean 67 g) in weight, were collected from Tiantai County, Zhejiang Province, and were bred in our snake garden. From March 1984 to December 1986, 178 females (about 5 females per month) were investigated. Each snake was measured in total length (SVL+TL) and total weight. The number and the size of follicles or embryos, as well as the weight of the ovaries and fat bodies, were examined by dissection. The pH value, whether sperms were stored up or not and the sperms' activity in the oviducts were determined too. All the above were done in order to understand seasonal variation of sex glands, ovulation and mating. From 1984 to 1987 in the gestation season (June, July and August) 82 mature female snakes were operated on and 731 embryos were obtained. The conditions of embryonic development were based on the studying of the external morphology of the embryos removed from the gravid females at regular intervals throughout the gestation period. Another 10 gravid females were bred apart to observe their rate of reproduction.

Results

The Female Reproductive Cycle

1. The seasonal variation of ovary weight. The ovary weight is expressed by

† This publication combines material previously published in Chinese by Huang et al. (1990) with additional discussion.

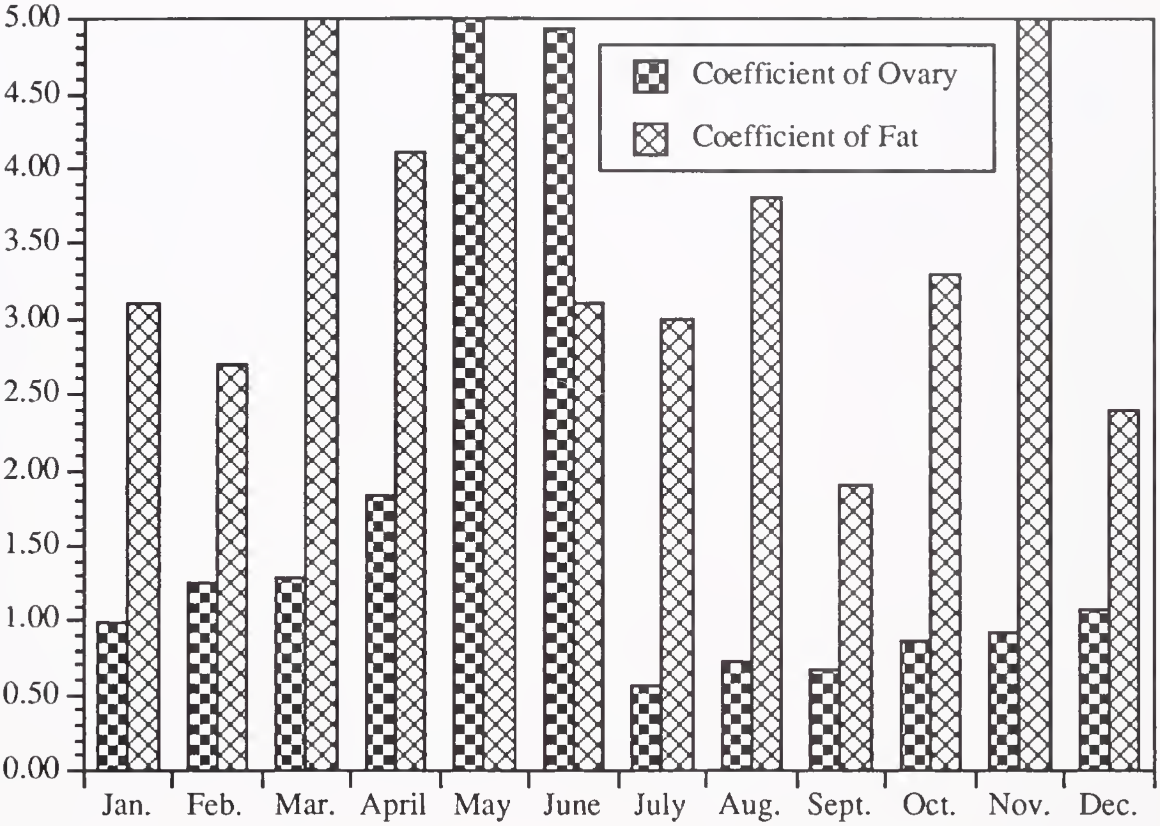

FIG. 1. Seasonal variation of ovary and fat body weight in *Agkistrodon blomhoffii brevicaudus*.

multiplying the coefficient of ovary (the ratio of wet weight of a pair of ovaries to that of total body weight of each individual) times 100 to take the form of a percentage. Figure 1 shows the seasonal variation. The eggs of the ovary were transferred to the oviduct in mid June. The coefficient of ovary reached the lowest point in July and then increased gradually. From late March to April in the next year, with vitellogenesis occuring, the coefficient of ovary rose obviously. It reached its top value in May or June.

II. The seasonal variation of fat bodies. The weight of fat bodies is expressed by multiplying the coefficient of fat (the ratio of the wet weight of fat body to that of the total body weight of each individual) times 100 to take the form of a percentage. Figure 2 shows its seasonal variation with two peaks in March and November. There was an inverse correlation between vitellogenesis and fat body size. Fat bodies enlarged in spring, reduced in September (after parturition), and then increased gradually until November (before hibernation).

III. The seasonal variation of follicle types. Developing ovarian follicles were divided into three classes by their size and location. Previtellogenic follicles were designated as class 1, vitellogenic ovarian follicles as class 2, and oviductal eggs as class 3. The Chinese Mamushi (*A. blomhoffii brevicaudus*) is a species with annual snake reproduction. All the mature females have class 1 follicles with a diameter of 0.5 to 10.0 mm, with a transparent or white color and round or oval shape. In April class 1 follicles were at their maximum in number. On the average, there were 25 class 1 follicles per female. The larger the size of the female, the larger the number of class 1 follicles it had. The largest female was 680 mm in length and 120 g in weight, and had 69 previtellogenic follicles. In spring,vitellogenesis began and follicles grew rapidly. Some of the class 1 follicles

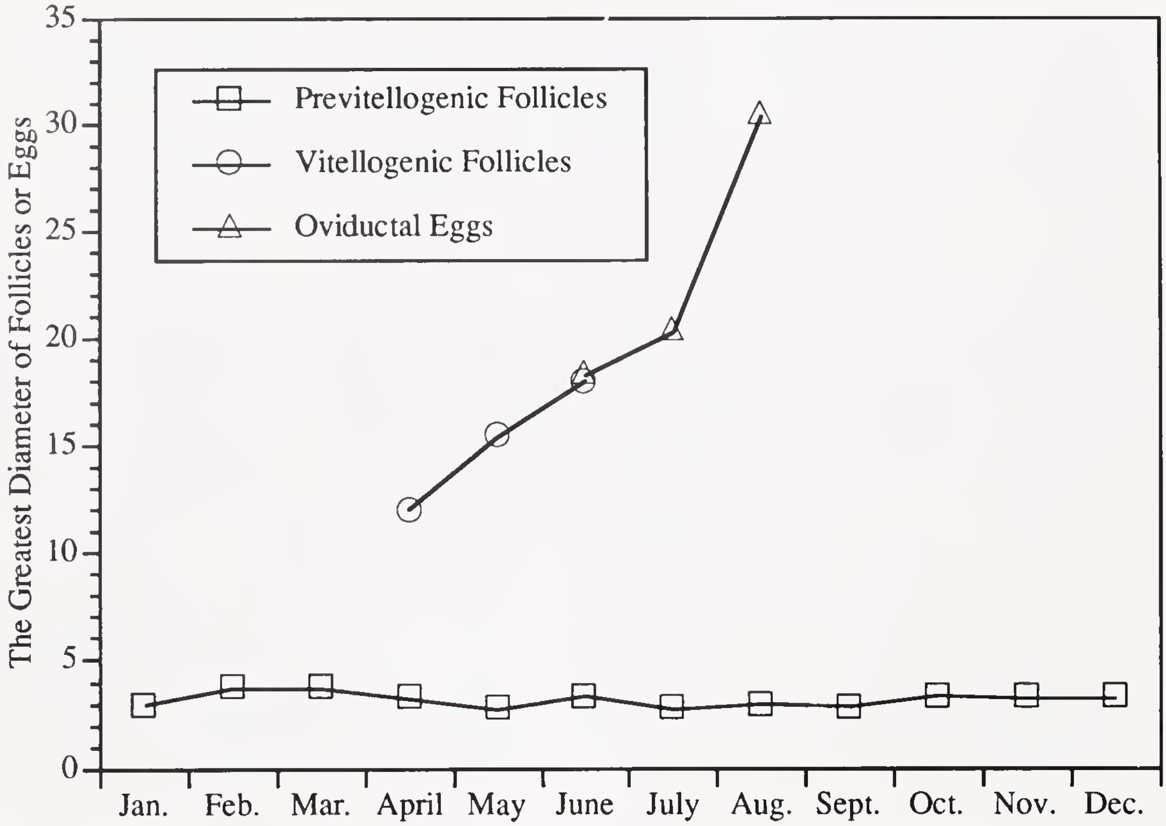

FIG. 2. Seasonal variation of follicle and egg size in *Agkistrodon blomhoffii brevicaudus.*

were transformed into class 2 follicles with yellow color, oval shape, and long diameters between 11.0-25.0 mm. They only existed from April to June. When follicles reached the largest value in about middle June, ovulation occured. The date of the earliest ovulation took place on June 16. Class 3 eggs were present in the oviducts from middle June to late August. The maximum diameter of an egg was 28.0 mm. The pH in the oviducts was 7.26±0.6

Embryonic Development

The development of the external morphology is described as follows. In middle June ovulation occurred, and eggs transferred to the oviducts. Fertilization and development took place. A small oval blastodisk divided on the large ellipsoidal yolk mass to form the blastula. Then gastrulation proceeded to form a blastopore marking the posterior end of the embryonic shield (Huang et al., 1989, stages 1-3). In late June the neural plate raised and folded. The blastopore was still visible. Then the neural groove appeared and expanded at the cephalic end. The amniotic fold was sharply raised anteriorly toward the head and covered more than half the embryo. After that the posterior amniotic fold was beginning to cover the tail bud. The neural tube was formed by the fusion of the neural folds. The amnion was closed. The allantois bulged slightly and was not inflated. The first rudiments of the heart and optic vesicles were formed (Huang et al., 1989, stages 4-9). In early July the allantois began to inflate. The heart took a "U" shape and began to beat. The caudal part of the embryo began to coil. Lans placodes invaginated. The mandibular segment and the auditory pits were visible. Branchial clefts were formed with the appearance of the nasal pits. The eye was lightly pigmented. Furthermore, the heart was shaped like an "S". Then both the ventricle and auricle were distinguishable. The cloacal mound was visible and the rudiment of the hemipenes appeared. The

TABLE 1. Reproductive data on 10 adult female *Agkistrodon blomhoffi brevicaudus.*

Adult Females					Length (mm) of young at birth			Weight (g) of young at birth		
No.	Length (mm) Vent+ Tail	Weight (g)	Date of parturition	Clutch size	max.	min.	ave.	max.	min.	ave.
86-8091	570 (510+60)	83.0	86.9.9	5	147+26	133+21	140.8+23.0	3.9	2.6	2.74
86-8092	640 (575+65)	137.7	86.9.9	5	152+25	142+25	145.7+23.7	3.3	2.8	3.08
86-8093	680 (610+70)	176.7	86.8.23	12	159+24	151+24	155.8+24.6	4.1	3.8	3.86
86-8094	610 (545+65)	203.7	86.8.26	20	158+27	132+22	143.5+22.1	3.8	2.6	2.96
86-8095	630 (560+70)	193.7	86.8.20	14[1]	145+25	119+18	132.9+20.7	4.1	1.6	2.61
86-8096	605 (540+65)	116.3	86.8.20	5[2]	175+28	165+28	170.0+28.0	5.3	5.0	5.10
88-8097	537 (475+62)	97.6	86.9.6	10	138+23	132+25	134.6+22.4	2.6	2.0	2.24
86-8098-	645 (575+70)	157.0	86.9.9	8	144+21	136+21	140.6+21.5	3.0	2.8	2.84
86-8099	555 (500+55)	147.3	86.8.18	6	163+23	146+22	150.8+22.0	3.6	3.2	3.45
86-8100	602 (535+67)	114.0	86.8.28	7	145+22	130+24	135.8+22.7	3.0	2.2	2.61

1- Nine still born. 2- One still born.

trunk coiled 4.5 circles (Huang et al., 1989, stages 10-11). By middle July the hemipenes became vesicle-like projections. The upper and lower jaws were visible and the trunk loosened into four circles (Huang et al., 1989, stage 12). In late July the hemipenes were blunt fork-like projections. The tongue was visible. The mid-line of the ventral body was enclosed except in a small circular area. Scales appeared on the trunk but not on the head. The trunk loosened further and coiled only 3-3.5 circles (Huang et al., 1989, stage 13). During early August scales on the trunk appeared to be keeled and their pigment pattern was well developed. Scales and pigmentation on the head were visible but the pattern was not well developed. The whole mid-line of the ventral body was enclosed. The hemipenes were still everted. The trunk coiled 2-2.5 circles (Huang et al., 1989, stages 14-15). In middle August the pigment pattern fully developed. The hemipenes were inverted in all the male specimens. Just prior to parturition, the embryo showed all the morphological characteristics of its own family. (Huang et al., 1989, stage 16).

Reproduction

The gestation period lasted for about 65-75 days and parturition occurred in middle to late August or September. Table 1 shows the state of the reproduction of 10 gravid females. Ten gravid females produced 10 litters which included 97 juveniles. The mean number per litter was about 10. Among 10 litters of new babies, about 99% had survived except one litter which had 9 stillbirths. All the live babies were 154 to 203 mm in length and 2.0 to 5.3 g in weight.

Discussion

I. According to Saint Girons (1982), the reproductive cycle of male snakes has four major types: 1) aestival (summer) or postnuptial type; 2) mixed type; 3) prenuptial type; and 4) continuous reproductive activity type. The reproductive cycle of the Chinese Mamushi (*A. blomhoffii brevicaudus*) belongs to the first type (Lin et al., in press), which is found only in temperate and subtropical regions. Spermatogenesis begins in spring after hibernation and spermiogenesis begins in summer. The spermatozoa are stored throughout the winter in the epididymis and vas deferens of the male or in the oviducts of the female. According to Saint Girons (1966), the reproductive cycle of female snakes has annual types A-F-G and biennial types B-C-D. By our observation the Chinese Mamushi (*A. blomhoffii brevicaudus*) is thought to be the annual type F. Its mating season is spring (April or May) and ovulation occurs regularly in middle June, while spermatogenesis is the aestival type. Sperm are stored in winter in the vas deferens of the male or in the female oviducts if there is fall mating. Sperm of the Chinese Mamushi (*A. blomhoffii brevicaudus*) may be stored in the oviducts and kept available for about three years (Hu et al., 1966). This characteristic is of great benefit to the survivialship of the Chinese Mamushi (*A. blomhoffii brevicaudus*).

II. Several investigators have found an inverse correlation between reproduction (vitelogenesis) and fat body size in snakes (Seigel and Ford, 1987). In snakes with annual reproduction (e.g. *Opheodrys aestivus*), fat bodies enlarge in spring, reach a low point in early to middle summer (egg-laying) and then increase gradually until hiberntion. In species with biennial or triennial reproduction, e.g. *Vipera berus* and *Crotalus viridis*, fat body reserves are lowest at the time of parturition (Macartney and Gregory, 1988; Seigel and Ford, 1987). Seasonal variation of fat bodies of the Chinese Mamushi (*A. blomhoffii brevicaudus*) is similar to the above observation. There are two peaks in March (spring) and November (hibernation). The low point is in September (at the time of parturition).

III. The sexual maturity of the Chinese Mamushi (*A. blomhoffii brevicaudus*) is attained in 2-3 years. Jin et al. (1983) mentioned that vitellogenesis began in the second spring after birth (20 months old), 350-484 mm long and 20-60 g in weight. In the Lined Snake (*Tropidoclonion lineatum*) from St. Louis, Missouri, USA, the same result was obtained by Krohmer and Aldridge (1985). All the females in our experiment were mature with a length of more than 447 mm and a weight of over 31 g each. There was a positive correlation between the number of yolking follicles or embryos and female length. The average number of embryos from 82 females was ten. Seventy one percent of the females 400-500 mm long contained less than ten embryos, while 75% of the females 500-600 mm long contained more than ten embryos.

IV. The Chinese Mamushi is ovoviviparous, which is evolved from oviparity. It is said to be an adaptation to variable environrnents where stochastic events jeopardize egg survivorship. But Seigel and Ford (1987) thought it also had some disadvantages, including lower clutch frequency, more mortality risks to the parent, less intake of food and higher metabolic cost of the parent. The clutch mass of ovoviviparous snakes is smaller than that of oviparous snakes. The physiological costs of reproduction (heart rate and oxygen consumption) significantly increased during pregnancy in ovoviviparous snakes. We found that pregnant snakes cease feeding during the last period of gestation, so they were thin and weak after parturition. If they did not gain enough food in time, they would be dead during hibernation or by the next spring. However we think that ovoviviparity is more favorable in protecting the filial generation because the survival rate is much higher in the Chinese Mamushi (*A. blomhoffii brevicaudus*). In our experiment the survival rate reached 90-99%.

Acknowledgments

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