Reproductive Behavior in the Japanese Salamander *Hynobius retardatus*

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Abstract: The reproductive behavior of the Japanese salamander, *Hynobius retardatus*, was observed in the laboratory. The sequence of reproductive behavior was: 1) males grasped twigs in the water and the female hid herself beneath a broad leaf on the bottom, 2) the female began to swim restlessly, 3) males turned their heads toward the female, 4) the female grasped the twig, 5) the female pressed her vent against the twig, 6) males turned their heads toward the female's vent, 7) the female rubbed her vent over the twig, 8) males brought their snouts close to the female's vent, 9) the female elevated her tail, 10) the male grasping the upper position of the twig held the female's waist, 11) the female began egg-sac laying, 12) the male pulled egg sacs out of the female's vent, 13) other males began to scramble for egg sacs, 14) the female fell to the bottom and hid herself beneath a leaf, 15) males ceased to hold egg sacs, and 16) males paid no attention to the egg sacs. On an average, the males required 571.3 sec for the behaviors from 3 to 15, and the females required 68.5 sec for the behaviors from 4 to 14.

Key words: Hynobiidae; *Hynobius retardatus*; Breeding; Behavior; Reproduction

In contrast to the majority of anurans, most of the urodeles fertilize eggs internally. In primitive salamanders of the families Cryptobranchidae and Hynobiidae, however, fertilization is external. Internal fertilization is assumed to have evolved from external fertilization in salamanders (Dunn, 1923; Arnold, 1977), but the details of this transition is still poorly understood. This is partly because reproductive behavior of primitive salamanders is still poorly known, as noted by Nussbaum (1985, 1987) and Verrel (1989). They suggested that the mating system and parental care of the primitive genera such as *Andrias* and *Hynobius* would provide important keys to understand the evolutionary relationships of the reproductive system between primitive and advanced salamanders.

Hynobius retardatus is endemic to Hokkaido, and is widespread throughout this island. Sasaki (1924) reported the spawning habits of this species, but his description is not sufficient to understand its reproductive system. The present study was undertaken to clarify the reproductive behavior and its temporal pattern in *H. retardatus* as a member of the primitive salamanders.

MATERIALS AND METHODS

Sexually active males and gravid females of *H. retardatus* were collected from breeding pools in

the basin of the Tottabetsu River, Obihiro (location in Sato, 1989). Five males and two females in 1986, 50 males and 18 females in 1987, five males and two females in 1988, 15 males and six females in 1989, and 30 males and 10 females in 1990 were collected from the field. In the laboratory, they were kept in a refrigerator (10°C) until the start of experiments in the following morning.

Two aquaria (60 × 30 × 36 cm) were used in the present study. The water level was 23 cm, and the water temperature was maintained at 10 ± 1°C in order to approach the conditions of spawning ponds in the field (the mean water temperature just beneath the water surface was 9.8°C (Sato, 1990)). Two twigs, ca. 70 cm in length and ca. 1.3 cm in diameter, were placed in an askew position, and broad leaves (e.g., *Quercus dentata* and *Q. mongolica* var. *grosseserrata*) were placed on the bottom of the tank. The length of the twigs was sufficient to allow five males to grasp them. All observations were performed under a LD 12 : 12 photoperiod, and the light intensity outside of the aquarium was 100 lux. The aquarium was illuminated by two 30-watt white lamps (total 40 lux) at an angle of 45° and 135° to record the reproductive behavior of the salamanders using a video camera (National VY-4700). These conditions did not seem to influence the reproductive behavior.

From field observations, it has been reported

that *H. retardatus* males holding a pair of egg sacs simultaneously is four to five in number and that no more than one female spawns at the same time on an object used for oviposition (Kawakami, 1963). Therefore, in the present study, five males and one gravid female were randomly chosen and placed in the aquarium. Five males were placed first at the same time and then one female was placed after each male found his own place in the aquarium. The conspicuous behavior was continuously observed all day long and recorded with the video camera from immediately before placing males till I removed the female one hour after she spawned. The experiments of the reproductive behavior were conducted 38 times and one male was used one or two times (an average of 1.8 times) in this experiment. The males used once and twice showed no detectable difference in reproductive behaviors. In order to identify the individual males, colored beads (ca. 0.02 g per piece) were attached to the back of the waist with fishing line (No. 0.5).

RESULTS

The egg-sac layings of 38 gravid females were successful and they began to oviposit ca. 22 hr (14 hr and 24 min to 26 hr and 55 min) after they were collected in the field.

Reproductive behavior before oviposition.—A typical sequence of the reproductive behavior before beginning of egg-sac laying is shown in Fig. 1, and the behavioral sequence and the time-interval (in sec) between starts of each behavior and onset of egg-sac laying are shown in Table 1. The five males swam vigorously just after being placed in the aquarium, but they stopped swimming in a few minutes. During this process, they showed one of the following three activity patterns: 1) all males grasped twigs; 2) four males grasped twigs and the remaining one walked around the bottom of the tank; 3) three males grasped twigs and the remaining two walked around the bottom. The majority (86.8%) was the second pattern. In the following description, mainly the behaviors of the second pattern were analyzed. The males other than the one walking on the bottom sometimes took a bite or poke at mouths, sides, backs or tails of other males suddenly and changed position relative to each other while grasping twigs. One male grasping the upper position of the twig sometimes showed a characteristic action in which he stiffened his body intermittently. The action became more frequent as the spawning of the female came close.

The first behavior of the female introduced into the aquarium was to hide herself beneath a broad leaf (Fig. 1-A) and to walk around the bottom slowly. The second behavior she exhibited was restless swimming (Fig. 1-B). The males did not respond to the female's behavior till she began to swim and walk actively. The males began to turn their heads toward her when her restless behavior became frequent. The time-interval between turning their heads and the beginning of egg-sac laying was 47.0 sec (Table 1). The female grasped one of two twigs which the males had been grasping (Fig. 1-C) and then pressed her vent against it (Fig. 1-D). Simultaneously, males on the twigs began to turn their heads toward the female's vent. Within 30 sec before the beginning of egg-sac laying, four males which had been looking at the female's vent brought their snouts close to it, and the male at the uppermost position straddled the female's back (Fig. 1-E). The female began to rub her vent over the twig and elevated her tail while arching her body and wagging her tail from side to side (Fig. 1-F). Just before the beginning of egg-sac laying, the male on her back held the female's waist with his forelimbs.

In three of 38 cases, the male that had been grasping the lower position swam down from a twig pursuing the female and straddled her tail at the bottom of the tank. At that time, she shook his body off her tail (ca. 867 sec before the beginning of egg-sac laying). In two cases, one male nudged the female's vent with his snout (16.0 sec before the beginning of egg-sac laying) and scratched her vent with his right forelimb in one case (9.0 sec before the beginning of egg-sac laying).

The mean water depth where egg sacs were attached was 4.1 ± 0.5 cm ($N=29$, Range, 0–9.0 cm).

Reproductive behavior after oviposition.—A typical sequence of reproductive behavior after the beginning of egg-sac laying is shown in Fig. 2, and the temporal sequence of behaviors is summarized in Table 1.

Immediately after the beginning of egg-sac laying, one male which had held the female's waist by his forelimbs grasped the base of the egg sacs with his forelimbs and pushed the female's vent with his hindlimbs (Fig. 2-A). However, in six cases, one male grasped the female around her vent with his forelimbs and pushed the base of the egg sacs with his hindlimbs. Apparently, the egg sacs were pulled out from the female's vent by this male's behavior (ca. 8 sec after the beginning of egg-sac laying; Table 1). It was, however,

FIG. 1. Typical sequence of the reproductive behavior of *Hynobius retardatus* before oviposition: A. Four males (non-shaded) grasp two twigs and one walks around the bottom of the tank, and one gravid female (shaded) hides herself beneath a broad leaf. B. Males do not show apparent interest in female's behavior till she begins to swim and walk actively. C. Males begin to turn their heads toward her. The female grasps the twig which males have been grasping. D. The female attaches her vent to the twig and rubs her vent over the twig. E. Males which have been looking at the female's vent bring their snouts close to it. F. The female elevates her tail arching her body and the male who has been grasping the upper position of the twig holds her waist with his forelimbs just before beginning of oviposition.

confirmed that the egg sacs could be pulled out without male's forelimbs or hindlimbs when the males stole the egg sacs from each other (in seven

cases). The other males, except the one looking upward on the bottom, also began to scramble for the egg sacs and simultaneously emitted their

FIG. 2. Typical sequence of reproductive behavior of *Hynobius retardatus* after oviposition: A. One male grasps the base of egg sacs with his forelimbs and then pushes the female's vent with his hindlimbs. B. Four males, except the one looking upward on the bottom, begin to scramble for the egg sacs and simultaneously emit their sperm. C. The male looking upward at the bottom suddenly begins to swim actively. After a while, the female ends egg-sac laying and leaves her egg sacs. D. The female hides herself beneath a broad leaf on the bottom again. Males grasp the twigs again and pay no attention to the egg sacs.

sperm (Fig. 2-B). On the other hand, the male looking upward at the bottom suddenly began swimming actively (15 sec after the beginning of egg-sac laying; Table 1) and almost joined the scrambling males. The maximum number of males scrambling simultaneously was one male in one case, two males in five cases, three in 12 cases, four in 19 cases and five in one case.

The female ended egg-sac laying ca. 24 sec after beginning. When two males were on the bottom, the one nearer to the egg sacs swam more quickly than the other and joined the other males in emitting sperm. Immediately after leaving the egg sacs, the spent female fell to the bottom arching her back and remaining motionless for an average of 26.6 sec (Fig. 2-C). The female began to move again ca. 51 sec after the beginning of egg-sac laying. She soon hid beneath a broad leaf (in 32 cases). On the other hand, males continued holding egg sacs for 280

to 687 (an average of 524.3) sec after the beginning of egg-sac laying (Fig. 2-D). They ended holding and sperm ejaculation, grasped the twigs again, and sometimes stiffened for a moment. After that they did not look at the egg sacs again or protect the egg sacs against other males.

DISCUSSION

In order to understand the evolution of the mode of fertilization that is crucial to clarify evolution of the order Caudata, it is necessary to accumulate knowledge of reproductive behaviors among salamander species that fertilize externally. For this purpose, description of reproductive behaviors should be made with detailed temporal properties so as to make interspecific comparisons more accurate. In the present report, I clarified the temporal relationships of each reproductive behavior for the first time among the hynobiid salamanders. Unfor-

TABLE 1. The reproductive behavior in *Hynobius retardatus* before and after oviposition. Behavioral sequence and time intervals (in sec) between start of each behavior and beginning of oviposition are given. 'N' shows the number of the experiment.

Behavior	Time interval				
	Sex	$\bar{x}$	SE	Range	N
Turn the heads toward the female	Males	47.0	4.2	21–102	28
Grasp the twig which males are grasping	Female	44.3	4.1	15–121	38
Press the vent against the twig	Female	35.3	3.9	10–111	38
Turn the heads toward the female's vent	Males	35.1	3.9	11–100	25
Rub the vent over the twig	Female	24.5	2.4	7– 70	38
Bring the snouts close to the female's vent	Male	20.7	1.9	6– 48	27
Elevate the tail while arching the body	Female	13.0	2.1	0– 51	38
Hold the female's waist	Male	1.3	0.2	0– 5	31
Begin egg-sac laying	Female	0		0	38
Pull out egg sacs from the female's vent	Male	7.8	1.3	1– 30	25
End egg-sac laying	Female	24.2	2.9	6– 85	36
End holding egg sacs	Males	524.3	15.5	280–687	34

tunately, the present results can not be compared with the other species at present because no other data on temporal patterns are available, and I must await future studies. In the following discussion, only some reproductive behaviors observed in *H. retardatus* are compared with those previously reported for other hynobiids.

The reproductive behaviors in *H. retardatus* have been described roughly in the laboratory (Sasaki, 1924) and in the field (Kawakami, 1963). Sasaki (1924) did not fully describe the behavior of both sexes just before beginning oviposition, but the fundamental patterns seem similar to those observed in the present study. Accurate time intervals between the reproductive behaviors of *H. retardatus* were not mentioned in Sasaki's (1924) report nor in other studies of Hynobiidae and Cryptobranchidae. Consequently, the present report is the first one to analyze the temporal pattern of the reproductive behavior.

When the gravid female started to swim, males suddenly bit and poked the other males, and these actions became more frequent as the spawning approached. Similar behavior was observed in *H. nebulosus* (Hayashi, 1980) and *Salamandrella keyserlingii* (Bassarukin and Borkin, 1984), and therefore, restless swimming of the gravid female seems to cause the male's aggressive behavior. In addition to biting and poking, wagging the tail, which is also regarded as an aggressive action against invaders, was reported in *H. takedai* and *H. nebulosus* (Tanaka, 1986, 1989). The territorial behavior reported in *H. takedai* and *H. nebulosus* (Tanaka, 1986, 1989) was not ascertained in *H.*

retardatus, nor was the "tail swinging" behavior reported in *S. keyserlingii* (Hashimoto, 1976; Bassarukin and Borkin, 1984; Nakabayashi et al., 1986). Since the tail swinging, different from "tail wagging", has never been reported for any species of *Hynobius* whose reproductive behavior was examined (Mashiba, 1969; Kuramoto and Kawaji, 1973; Hayashi, 1980; Tanaka, 1986, 1989), the behavior may be restricted to the genus *Salamandrella*.

The male, while grasping the twig, sometimes stiffened his body intermittently and the action seems to be related to sexual excitement of males, since this action became more frequent as the spawning approached. A similar action has been reported in male *H. nebulosus* (Kuramoto and Kawaji, 1973; Hayashi, 1980), *H. dunni* (Mashiba, 1969) and *S. keyserlingii* (Takayama, 1978). In my observation, the stiffening of the body was sometimes observed even after males emitted sperm, and thus sexual excitement of the males seem to last for some time. When the female's restless behavior became frequent, males turned their heads toward her, and went even closer to her when she was ready to oviposit. Males of other species are reported to show a series of behaviors similar to those found in *H. retardatus* (Sasaki, 1924) and this male behavioral series seem to be general in the hynobiid salamanders.

"Midwifing" behavior, in which the male grasps the egg sacs and shoves the female backward to assist the delivery of the egg sacs, was confirmed in the present observations (Fig. 2-A). This behavior was confirmed clearly only in *H. retardatus* (Sasaki, 1924; Sato, this study), whereas the other hynobiid male scrambled for

egg sacs after the birth of the egg sacs (Mashiba, 1969; Kuramoto and Kawaji, 1973; Nakabayashi et al., 1986). Sasaki (1924) considered that midwifing behavior is an unselfish behavior designed to assist the female in the delivery of eggs, but Nussbaum (1985) regarded it a result of sexual selection among males to hasten the delivery of eggs so that males are in a good position to place spermatozoa directly on the eggs.

In the present study of *H. retardatus*, male-male aggression was not confirmed during scrambling for egg sacs. This conforms to Sasaki's (1924) observation of the same species, but contrasts to *S. keyserlingii* (Hashimoto, 1976; Bassarukin and Borkin, 1984). Whether or not the presence of aggression during fertilization is unique to the genus *Salamandrella* is unknown, and additional observations of other *Hynobius* species is necessary to compare these genera. I could not observe parental care in *H. retardatus*. Although Thorn (1962) reported that male *H. nebulosus* take care of egg sacs, the report was doubted by Nussbaum (1985). Thus, the parental care, reported to occur in the majority of salamanders (Duellman and Trueb, 1986), has not been ascertained in the hynobiid species.

After laying the egg-sacs the female hid herself beneath a broad leaf in most cases. In the field, Kawakami (1963) and Kuramoto and Kawaji (1973) observed spent females of *H. retardatus* and *H. nebulosus*, respectively, hide under stones or leaves in the field. Thus, resting immediately after oviposition seems usual in the *Hynobius* females. Moreover, I observed a female *H. retardatus* eaten by crayfish immediately after oviposition (Sato, 1991). The female after oviposition is slow in movement and is apt to be attacked by enemies because she has exhausted much energy for spawning, and probably hiding immediately after oviposition will be crucial for her to defend herself against natural enemies and recover her strength.

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要旨 エゾサンショウウオにおける生殖行動

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エゾサンショウウオの生殖行動を研究室内で観察した。生殖行動の順序は以下のとおりであった。1) 雄は枝につかまり、雌は水底の葉の下に隠れている。2) 雌は落ち着きなく泳ぎ始める。3) 雄は雌の方に向く。4) 雌は雄が待つ枝につかまる。5) 雌は枝に総排出口を付ける。6) 雄は雌の総排出口の方に向く。7) 雌は枝に総排出口を擦り付ける。8) 雄は雌の総排出口に吻部を近付ける。9) 雌は体を曲げながら尾を上げる。10) 枝の最上位にいた雄が雌の腰をつかむ。11) 雌は卵嚢を産み付け始める。12)

雌の腰をつかんでいた雄は雌の総排出口から卵嚢を引き出す。13) 複数の雄が卵嚢を奪い始める。14) 雌は水底に降り、葉の下に隠れる。15) 雄は卵嚢を抱くのをやめる。16) 雄は卵嚢に関心を示さなくなる。雄では3) から15) までに平均571.3秒を要し、雌では4) から14) までに平均68.5秒を要した。

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