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Entering and Exiting Routes of *Hynobius leechii* to a Breeding Site and Staying Time within the Site

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ABSTRACT: To study entering and exiting routes of male and female *Hynobius leechii* to a breeding site and staying time of them within the breeding site, we monitored a breeding population located in the research forests of Kangwon National University. The breeding site was surrounded by a drift fence associated with nine pitfall traps. The breeding season of this population was about one month, from 16 March to 13 April, 2005. Breeding males arrived earlier at the breeding pond than females did. The operational sex ratio (OSR), defined as the ratio of males to females which are ready to mate, over a breeding season was female-biased as 0.67 male vs 1 female (57 males vs 87 females), but daily OSRs, OSR in a particular day, within the breeding pond were male-biased with 1.36~7.5 male vs 1 female in six days out of seven investigated days. While breeding males stayed in the breeding pond for about 11 days, breeding females left the pond as soon as they completed oviposition. However, the females stayed at terrestrial areas near the pond for about seven days before completely leaving the breeding site. Entering and exiting routes to the breeding site were different between males and females, and between ovulated and oviposited females. Both males and females arrived earlier at the breeding site stayed longer within the site. Males stayed longer within the breeding site lost more body weight.

Key words: Daily OSR, *Hynobius leechii*, Operational sex ratio (OSR), Salamander

INTRODUCTION

In Korea six salamander species have reported to date. They are *Hynobius leechii*, *H. quelpartensis*, *H. yangi*, *Onychodactylus fischeri*, *Salamandrella keyserlingii* in family Hynobiidae and recently reported *Karsenia koreana* in family Plethodontidae (Yang *et al.* 2001, Kim *et al.* 2003, Min *et al.* 2005). Most studies done in Korea have focused on determining the distribution of salamanders in specific areas such as a national park (Yang *et al.* 2001). Recent systematic studies of Korean salamanders resulted in the enrollment of two new species of *H. yangi* and *K. koreana* (Kim *et al.* 2003, Min *et al.* 2005).

Understanding various behavioral and ecological aspects of a salamander species is a key factor to successfully conserve or rehabilitate the species (Verrell and Halliday 1985). Only one field study of a breeding population for Korean salamanders was conducted, where they reported the breeding season of the population was about one month from early March to April (Park and Park 2000). The operational sex ratio (OSR), defined as the ratio of males to females which are ready to mate within a breeding population (Clutton-Brock and Vincent 1991), of the population was male-biased as 3.8 male vs 1 female. After entering to the breeding site, breeding males stayed for a while within the site, while females left the site just after oviposition. Multiple males participated in the external fertilization of eggs from a female when she was attaching egg sacs to the substrate such as pebble, water plant, tree twig, or dried plant stem (Park *et al.* 1996, Park and Park 2000). Considering that only few studies have conducted in the areas of breeding and behavioral ecology, more studies in these areas are necessary.

To date, no information is available about which routes *H. leechii* uses for entering or exiting a breeding site, whether or not males and females use the same routes, and how long males and females stay within a breeding site. To study entering and exiting routes of salamanders to a breeding site and staying time within the site, we investigated the dynamics of a breeding population located in the research forests of Kangwon National University, where we used a drift fence census method associated with pitfall traps.

MATERIALS AND METHODS

Study Site

The breeding site, a small pond, is located in the research forests of Kangwon National University, Bongmyeong-ri, Dongsan-myeon, Chuncheon-shi, Kangwon-do (37°46'19", 127°48'56"). The pond is within a valley adjacent to the foot of a mountain, which is connec-

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ted to a small mountain stream through a concrete tunnel of 4.1m length. The pond is oval with 2.1m in the shortest diameter, 2.8m in the longest diameter, and 28cm in its depth. The bottom substrate of the pond is mud with 26cm depth. Dried plant twigs and leaves of *Castanea crenata* were used as hiding places for salamanders. Dried plants of *Persicaria thunbergii*, *Erigeron annuus*, *Impatiens texori*, and *Oenanthe javanica* stand around the edge of the pond. About one third portion of the pond is faced to the mountain, about one forth portion is to a grass field, and the rest of the pond is to an unpaved mountain road of 2.35m width (Fig. 1). The pond receives underground water from the mountain and the water flow out to the stream. No dry of the pond occurred at least for last two years.

Monitoring a Breeding Population

To capture entering and exiting salamanders to a breeding pond, we constructed a drift fence associated with nine pitfall traps surrounding the pond on 18 March 2005, which are about 1m distance from the edge of the pond. Drift fence is made of white Formax (30cm×180cm, GS chemical). To prevent salamanders from moving through the bottom of the fence, we buried the fence at 10cm depth. In case of raining, to allow the water to flow through the fence, more than 1,000 holes (diameter 0.8cm) were made at the bottom of the fence.

Total nine rectangular pitfall traps (23cm×15cm×15cm), four both inside and outside the fence and one in the waterway of underground water, were buried and adhered to the fence (Fig. 1). The trap entrance was leveled to the ground surface so that salamanders easily fell into the traps. We filled each trap with water at 5cm depth to prevent salamanders caught by the traps from drying and also placed several leaves and two plat pebbles to provide a shelter for avoiding predation.

Total 16 field surveys were done from 16 March to 13 April, 2005. Daily survey was made between 1600 and 2300. During a survey, we checked out each pitfall trap at different times (1~4 times a day) to determine if salamanders were caught. Furthermore, we counted all individuals within the breeding pond in 13 surveys. Throughout the study period, we measured air temperature, humidity, and water temperature to 0.01 units using an electric thermometer (RS-232, Center, Taiwan) and an Hg thermometer on each survey day. We had five raining days each in March and April. Precipitation data were obtained from the Kangwon Regional Meteorological Office (http://gangwon.kma.go.kr/fweat/sanup.php) because the study area is close to the site (~25km) where the office provides meteorological data for Chuncheon-shi.

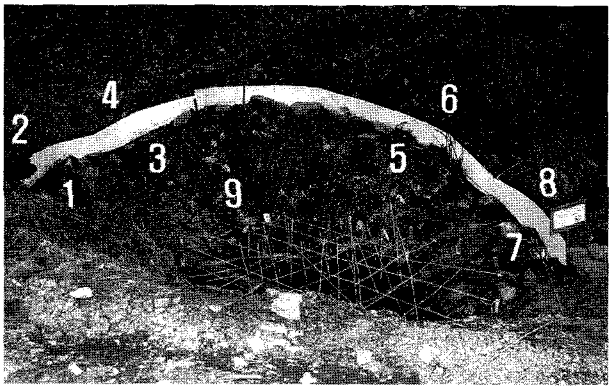

Fig. 1. A photograph showing a constructed drift fence and a pitfall trap.

We captured salamanders by bare hands or using a small fish net. We recorded for each individual captured day, time, location, and measured physical characteristics such as total length, SVL (snout-vent length), body weight, head length and width, and tail width on a plastic plate dish (23cm×15cm). In this paper, we did not present data about physical characteristics because of our previous report (Park *et al.* 1996).

After recording physical characteristics, we toe-clipped each individual for identification. After toe-clipping, we applied Pobidon (Green Pharmacy) to prevent salamanders from possible fungal or viral infection and preserved the clipped toes in 10% formaldehyde for later examinations. Although a recent study showed that toe-clipping somewhat increases the mortality of salamanders (McCarthy and Parris 2004), toe-clipping is still the most effective method for individual marking of salamanders (Funk *et al.* 2005).

We considered that an individual caught in a breeding pond is an earlier arriver at the breeding site than an individual caught in pitfall traps although both individuals were checked on the same day and the same survey time. In *Hynobius*, since an individual arrived at a breeding pond immediately changes their physical and physiological conditions for mating (Hasumi and Iwasawa 1992), such an entering order determination of salamanders seemed reasonable to compare physical conditions of individuals based on arriving time at the breeding site.

Salamanders caught in pitfall traps located outside the fence were considered as entering individuals to the breeding site so that we released them inside the fence. On the contrary, individuals caught in pitfall traps inside fence were released outside the fence, considering that they were exiting individuals. Individuals caught in the 9th traps placed on a waterway inside the fence were classified into ovulated or oviposited females based on the presence or absence of visible eggs in the abdomen and marked or unmarked males. Ovulated females and unmarked males were released inside the fence. Oviposited and marked males were released outside the fence. When we recaptured individuals, we recorded recaptured day, time, location, body weight, and individual number and then released them following the same protocol described above.

Statistics

We used Pearson's correlation analysis to analyze relationship between the number of males and females and environmental conditions, between entering order to the breeding pond and staying time within the pond, and between change in body weight of males and staying time within the pond (Sokal and Rohlf 1981). For relationship between change of body weight and staying time, we also produced a regression equation using regression analysis. The difference in staying time within the breeding site between males and females was analyzed by independent sample *t*-test because the data passed Shapiro-Wilk's normality test ($P > 0.05$). Chi-square test was applied to compare recapture rate between males and females and also to determine if males and females as well as ovulated and oviposited females use the same entering and exiting routes to a breeding site. Numerical data in the text were presented as mean ± SD.

RESULTS

Breeding Period and Operational Sex Ratio (OSR)

Over the study period, average air temperature was 14.12 ± 5.20 (n=15, range=7.0~22.0), average water temperature was 6.89 ± 0.97 (n=14, range=1.3~11.8), and average humidity was 49.71 ± 4.11 (n=13, range=26~72). Fig. 2 shows the number of males and females newly caught on each survey day associated with environmental conditions, in which only water temperature showed significant relationship with the number of individuals caught on a survey day (female; r=0.58, n=12, P=0.047, male; r=0.57, n=12, P=0.056). The breeding period of this population was about one month, from 16 March to 13 April. There were two breeding peaks on 22 March and 7 April based on the number of females caught on the days (Fig. 2). In genus *Hynobius*, females determine a breeding peak because females oviposite once, while males do multiple participation in mating.

More than 58% of total 58 males entered into the breeding pond in three days, 16 males on 18 March and 18 males on 7 and 8 April. Except these three days, less than 5 males arrived at the breeding pond in a day. Males arrived at the pond two days earlier than females. On 22 March, 11 females arrived at the pond, which resulted in the first breeding peak. Second breeding peak occurred when 11 and 17 females entered to the pond on 4 and 7 April.

Total 145 salamanders, 58 males and 87 females, were caught in this study. Fig. 3 shows size frequency distribution of the salamanders. The operational sex ratio of this population over the breeding period was female-biased as 0.67 male vs 1 female. However, daily

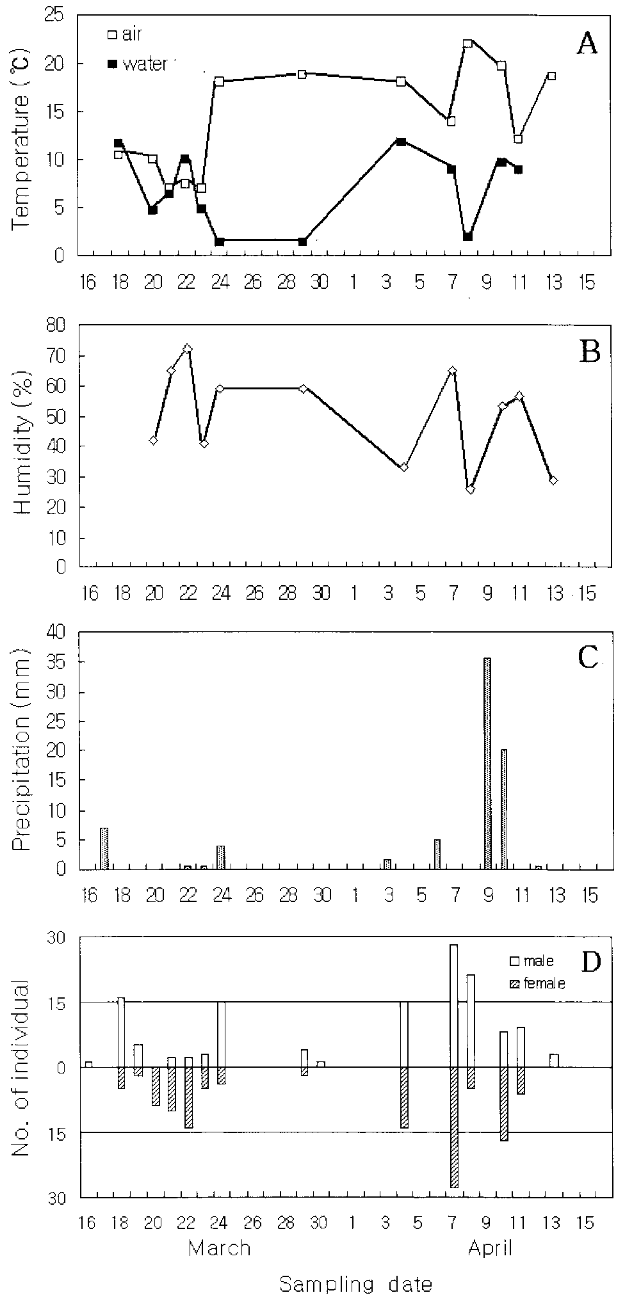

Fig. 2. The daily air and water temperature (A), humidity (B), precipitation (C), and the number of males and females caught in the breeding site on the survey days (D) from 16 March to 13 April, 2005.

OSRs, OSR in a particular day, were mostly male-biased. From total 13 surveys that checked all salamanders within the breeding site, we used seven survey data to calculate daily OSRs because in six surveys no females or no ovulated females were caught so that we can not obtain a daily OSR based on the definition by Clutton-Brock and Vincent (1991). Daily OSR was male-biased in six days out of seven survey days (3.26 ± 2.37, n=7), ranged 1.36 (15 males vs

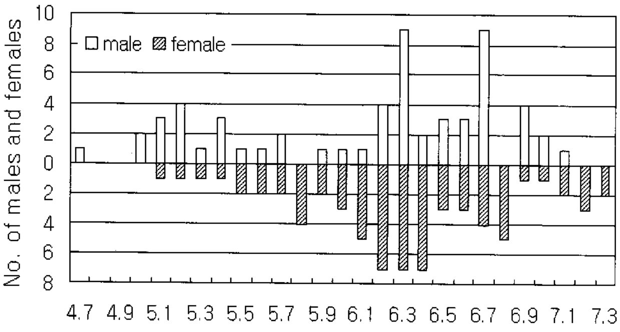

Fig. 3. The snout-vent length (SVL) - frequency histogram of males and females caught in the breeding site.

11 females on 4 April) to 7.5 (15 males vs 2 females on 24 March). Only one day, 10 April, daily OSR was female-biased as 8 males vs 9 females.

Entering and Exiting Routes to a Breeding Site

Total 152 salamanders, 56 males and 96 females, were caught in pitfall traps. Captured rate of males and females was not different (χ^2=0.92, *df*=1, *P*=0.34). Because we were interesting to know which entering and exiting routes to a breeding site male and female salamanders use, we included the individuals caught more than once in pitfall traps during the breeding period.

Twenty nine ovulated females were caught outside the fence and 11 inside the fence. Forty oviposited females were caught inside the fence and 2 outside the fence. Ovulated and oviposited females differentially used entering and exiting routes (χ^2=102.14, *df*=4, *P*< 0.001, Fig. 4A). Most ovulated females were caught in the 4th pitfall trap, located on the ground of an underground waterway or in the 9th trap in the waterway. Oviposited females were relatively evenly caught in the 1st, 3rd, and 5th traps. Only three oviposited females were found in the 9th trap.

Entering and exiting routes to the breeding site between males and females were also different (χ^2=13.30, *df*=4, *P*=0.0099, Fig. 4B). In this analysis, the number of females was the sum of ovulated and oviposited females. We found 16 males within the pond on 18 March, when we constructed the fence. Except them, most males were caught in the 9th trap placed in the waterway. Very few males were relatively evenly caught in other pitfall traps.

Staying Time within a Breeding Site based on Recapture Data

Recapture rate between males and females was different (χ^2= 37.87, *df*=1, *P*<0.001). Thirty four females out of 87 were recaptured, while 40 males out of total 58 were recaptured. Also, 22 males out of 40 recaptured males were caught again, while only three females out of 34 were caught again (χ^2=37.07, *df*=1, *P*<

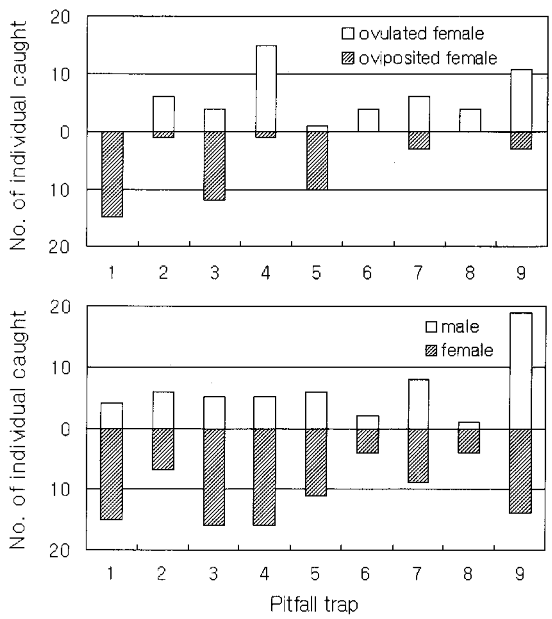

Fig. 4. The use of entering and exiting routes to a breeding site between ovulated and oviposited females (A) and between males and females (B).

0.001). Seven males were caught four times and three males five times. In addition, 28 out of 40 recaptured males were caught at least more than once within the breeding pond, but there was no female recaptured within the pond.

We calculated staying time of 40 males and 34 females within the breeding site using recapture data. Males stayed 10.63 days ± 7.62 (range: 1~24 days, *n*=40), and females stayed 7.59 days ± 6.90 (range: 1~20 days, *n*=34). Staying time between males and females was not different (*t*=1.78, *df*=72, *P*=0.08).

We analyzed relationships between entering order and staying time of 40 males and 34 females. Both males and females arrived earlier stayed longer within the breeding site (male: *r*=0.66, *n*=40, *P*<0.001, Fig. 5; female: *r*=0.55, *n*=34, *P*<0.001). Change in body weight with staying time within the breeding site was analyzed in males. In this analysis, we excluded females because of major body weight changes after oviposition. Males stayed longer in the breeding site lost more body weight (*r*=0.68, *n*=30, *P*<0.001, Fig. 6) and it was expressed as change in body weight = −0.059×(staying days within the breeding pond) − 0.117.

DISCUSSION

Our results showed that males and females as well as ovulated and oviposited females differentially used entering and exiting routes to the breeding site. Breeding males stayed in the breeding pond about 11 days after entering. Breeding females left the pond as soon as they oviposited eggs, but then stayed terrestrial areas near the

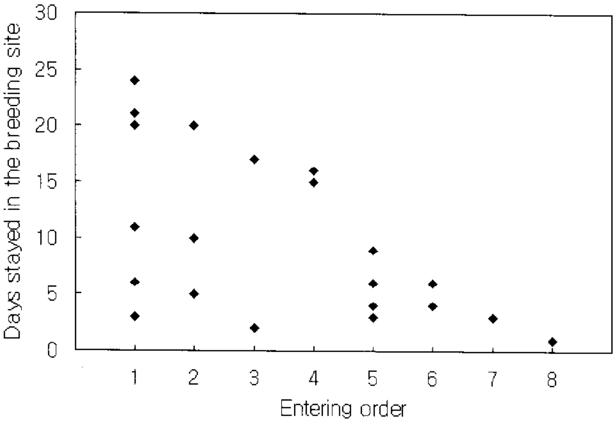

Fig. 5. Entering order of males at the breeding site showed a negative correlation with the number of days stayed in the breeding site. Nineteen data had the same values overlapped with the other data, resulting in 21 samples appeared in the figure, not 40 samples as described in the results.

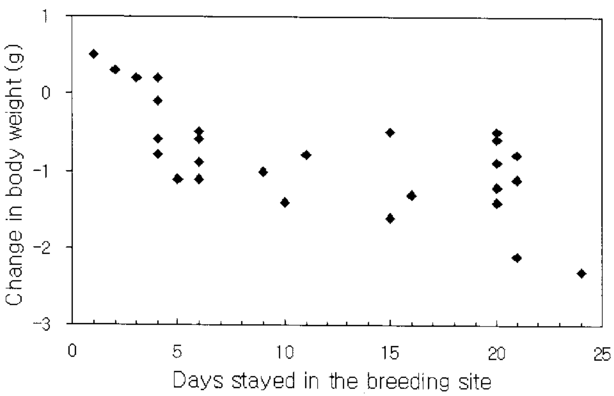

Fig. 6. Males stayed longer in the breeding site lost more body weight. Three data had the same values overlapped with the other data, resulting in 27 samples appeared in the figure, not 30 samples as described in the results.

pond about seven days before completely leaving the breeding site. Although OSR over a breeding season was female-biased, daily OSR within the breeding site was male-biased in most days. These results suggest that differential entering and staying pattern to a breeding site of males and females may be responsible for strong mating competitions observed in *H. leechii* (Park and Park 2000).

Breeding period of this population is similar to the previous result as about one month (Park and Park 2000). But, this population had two breeding peaks each in March and in April. Such separate breeding peaks were previously reported in the breeding populations of *H. nebulosus* and *Nothophthalmus viridescens* (Hurlbert 1969, Kusano 1980). The authors suggested that such a pattern was caused by a sudden change of environmental conditions which were favorable for breeding. Park *et al.* (1996) reported that *H. leechii* prefers 7~12 ℃ water temperature for breeding. In this study, only water temperature showed a positive relationship with the number of males and females entering to the breeding pond. Considering these results, decline of the first breeding peak may be caused by a sudden decrease in water temperature to 1℃, while the second breeding peak may be brought by recovered water temperature to 10℃.

Males and females showed different patterns of entering and exiting to a breeding site. Over one or two days, most males entered into the breeding site. Except those days, very few males entered to the breeding site in a day. While, females entering to the breeding site showed gradual increase and decrease over a whole breeding period. This entering pattern to a breeding site is similar to the previous result (Kim 2000, Park and Park 2000). After entering to the pond, males stayed about 11 days within the breeding pond before exiting, while females left the pond as soon as oviposition was complete, but then stayed about 8 days at terrestrial areas near the pond before completely leaving the breeding site.

Such a different pattern of entering, staying, and exiting to a breeding site between males and females may be a key factor to understand mating competitions among males within a population (Kokko and Monaghan 2001). The pattern of different entering and staying pattern between males and females was resulted in a male-biased daily OSR even though the OSR of this population was female-biased, suggesting that male-male mating competitions would be high in this population. In salamanders that internally fertilize eggs, the OSR of a population and daily OSRs within the population are similar each other although local OSRs are slightly different in different time and space (Kvarnemo and Ahnesjö 1996, Rohr *et al.* 2005). In such salamanders, females can store sperms within a specialized organ, spermatheca, from several days to more than a year (Sever *et al.* 1996). Females often participate in mating courtship several times for multiple mate, and receive sperms from several males over a whole breeding period (Halliday 1998), so that the number of females plays a part in daily OSRs over a period. On the contrary, in salamanders that externally fertilize eggs, females can not store sperms and participate in mating only once so that they can only play a part in a specific daily OSR, while male salamanders affect daily OSRs over a long period by staying within the breeding pond for a while and participating in multiple mating. Thus, to determine the intensity of mating competition in externally fertilizing salamanders, daily OSR should be a more useful tool than OSR over a breeding period. Our finding of male-biased daily OSRs suggests that high male-male competition to fertilize limited eggs from females would occur in natural populations of *H. leechii*.

Males and females used different entering and exiting routes to a breeding site. Most males followed a waterway to enter the pond, while females preferred a route on the ground where a waterway is under the ground. The use of the routes between ovulated and oviposited females was also different. Ovulated females preferred a route on the ground where a waterway is under the ground, but oviposited females oriented towards a grass field regardless of a waterway. The difference of entering or exiting routes between adults and sub adults of *N. viridescens* (Hurblert 1969) and between males and females and between adults and sub adults of *N. perstriatus* (Johnson 2003) have reported. But, authors did not determine if such a difference was caused by a different preference for feeding habitats where they headed or if they specifically preferred a particular route for entering and exiting to a breeding site. In *Hynobius*, Kusano and Miyashita (1984) showed that feeding habitats of males and females were not different. To elucidate what causes these entering and exiting route differences between males and females and between ovulated and oviposited females, further studies are needed.

In *Hynobius*, it was known that males stay for a while within the breeding area after breeding migration, while females immediately left the area after oviposition (Kusano 1980, Hasumi and Iwasawa 1992, Park and Park 2000). In this study, breeding females immediately left the breeding pond after oviposition, but stayed quite a time at terrestrial areas near the pond. Why are females staying at the area? One possible hypothesis to explain this feature is that females may stay in the areas to recover energy expenditure for oviposition. Considering that a long emigration of 100 to 500m (Kusano and Miyashita 1984) from a breeding site to a feeding habitat could be fatal for female salamanders without full recovery of health conditions.

Both males and females arrived earlier stayed longer within the breeding site. Particularly males stayed longer lost more body weight. Kusano (1980) reported that males allocate few time and energy to forage during a breeding period. Salamanders arrived early in the breeding site might have more chance to participate in early mating. In general, early oviposited salamander eggs have a higher chance to be successfully hatched and metamorphosed with low mortality (Boone *et al.* 2002). Studies are necessary to determine if salamanders arrived earlier at the breeding site participate in more mating and achieve a higher mating success than late arrivers.

In conclusion, our study first elucidated entering and exiting routes of *H. leechii* to a breeding site and increased our understanding about staying patterns of the species at the breeding site. In special, our data about the population and local operational sex ratios over a breeding period explain why *H. leechii* have a strong male-male competition. Finally, these field results could be applied in the conservation and/or rehabilitation of salamander populations in the field.

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