Site Selection for Hibernation by the Tree Frog, *Rhacophorus schlegelii*

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Abstract: Hibernation sites of the tree frog, *Rhacophorus schlegelii*, were studied in the Nobi District of Miura Peninsula, central Japan, in the winter of 1997–1998. Twenty six frogs were detected hibernating in upper layers ($\bar{x} \pm SD = 3.1 \pm 1.6$ cm in depth) of the soil at particular spots located at the edge of permanent ponds and in the banks of small streams at the headwaters. Soil conditions at the hibernating spots were characterized by low hardness, high water content, a lack of thick litter layer, and constantly cool temperature. In laboratory experiments, the frogs also showed clear preference for the soft and wet soil. Laboratory experiments together with field observations indicate that *R. schlegelii* selects particular places for hibernation in the soil to facilitate absorbing water and to reduce evaporative body water loss during dry winter on the Pacific coast of central Japan.

Key words: Anura; *Rhacophorus schlegelii*; Hibernation; Soil humidity

Terrestrial amphibians are known to take up water through the skin from the surrounding soil (Ruibal et al., 1969; Baldwin, 1974; Hillyard, 1976; Brekke et al., 1991; Griffiths, 1996). The high permeability of amphibian skin in turn makes amphibians susceptible to water loss by evaporation. Many anurans show various unique morphological, physiological, or behavioral adaptations to prevent body water loss (Duellman and Trueb, 1986; Stebbins and Cohen, 1995; Matsui, 1996). Burrowing into the soil for hibernation is usually interpreted as an adaptation for avoidance of freezing. Though Japan is located in the temperate zone with moderate annual precipitation, there is low precipitation for several months in winter in the Pacific coastal region of eastern Japan. Therefore, desiccation might be a problem for hibernating amphibians, but the way frogs prevent desiccation during hibernation has been never studied in Japan. Anurans inhabiting arid regions are reported to burrow into moist soil to escape from heat or desiccation of the surface soil (Creusere and Whitford, 1976; McClanahan et al., 1976; McDiarmid and Foster, 1987; Brekke et al., 1991). Moreover, there is a report that the spadefoot toad, *Scaphiophus hammondi*, burrows into soil to escape from winter dryness and to absorb water from the wet soil (Ruibal et al., 1969). Accordingly, it might be assumed that frogs overwinter in soil not only

for avoidance of freezing but also to taking up water from soil in the dry winter.

This study was carried out to discover the behavioral and ecological mechanisms for conserving body water in the tree frog *Rhacophorus schlegelii* during hibernation. This frog inhabits paddy fields and marshes at low altitudes in Honshu, Shikoku and Kyushu, breeds in spring, and digs a shallow hole in the mud to make a foamy egg nest at the edge of waterbodies surrounded by forest (Maeda and Matsui, 1989). Wada (1936) reported that the frog hibernates 10 cm below the ground surface in the marshes where it spawns.

In the present study, an attempt was made to discover how this frog prevents desiccation during hibernation. For this purpose, the relation between hibernation sites and soil conditions was examined in the field. Laboratory experiments were also done to examine how soil conditions such as hardness, humidity, and litter layer influence burrowing behavior of the frog.

METHODS

Study area.—The field study was conducted at Nobi (35°12' N, 139°42' E) of Yokosuka City on the Miura Peninsula located about 40 km southwest of metropolitan Tokyo, Japan. There is a hill there (116 m above sea level) with several streams and ponds. I selected a study area on the south slope of the hill. There are two small valleys there with streams and small ponds. The area is covered by broad-leaved

trees, dominated by *Lithocarpus edulis* at a ridge of the hill and by *Cinamomum japonicum* on the slopes and bottoms of the valleys. The area below the study area is mostly occupied by paddy fields, and is one of the few suitable habitats for *R. schlegelii* left in the Yokosuka region (Hayashi et al., 1990). Although hibernation of this frog has been observed in a paddy field in Aomori Prefecture, the northernmost prefecture of Honshu Island (Wada, 1936), hibernation on the forest floor has never been described for this species. This study was mainly carried out on the floor of broad-leaved forests surrounding a paddy field.

Capture of hibernating individuals.—A survey of hibernating frogs was carried out at 16 sites during the period from December 1997 to February 1998 (Fig. 1). Relatively gentle slopes were selected as study sites in order to facilitate the survey. The size of the study sites ranged from 150 to 200 m². At each site, the soil was dug up with a shovel to a depth of 10 cm from the surface, except for places with many rocks, logs, or tree roots. No attempt was made to find frogs in the water. Eventually, 28 days (average seven hours/day) were needed to dig up the 16 study sites.

When frogs were found during the survey, measurements were taken from the uppermost part of the frog body to soil surface. Frogs were brought back to the laboratory and snout-vent length (SVL) was measured with vernier calipers to the nearest mm.

Soil conditions at hibernation points.—Soil conditions were compared between points with and without frogs to discover common features of the soil conditions of hibernation points. The soil conditions adopted in this study are hardness, water saturation percentage, thickness of A₀ layer, and temperature. The measurements of these soil characters were taken in a particular area where many hibernating frogs were captured (Fig. 1, site A). At site A, hibernating frogs were observed at only two points. Therefore, the four points without hibernating frogs near the hibernation points were selected to compare the soil conditions (Fig. 2). The soil characters were measured at these six points in January 1998. Soil temperature was measured every two weeks from December 1997 to February 1998.

For measurement of the soil hardness, a quadrat (50×50 cm) with 25 compartments (10×10 cm) was placed at each point, and the hardness was measured with a hardness meter (Yamanaka type) at the center of each compart-

ment. Thickness of the A₀ layer was expressed as the total thickness of litter, fermentation, and humus layers. Water saturation percentage of the soil was measured with a water saturation instrument (YZ-132 Yazaki product) at the depth of 5 cm from the surface. Soil temperature was measured at noon at the depth of 3 cm from the surface.

Burrowing experiment.—Twenty six frogs collected at the hibernating sites were used as experimental animals to examine burrowing behavior under different soil conditions of hardness, moisture, and litter layer. Collected frogs were brought to the laboratory and divided into adults and juveniles. All adults collected on the same day were put into an experimental chamber, and all juveniles collected on the same day were put into another experimental chamber. The numbers of frogs in one chamber ranged from one to four. The experimental chambers were plastic cages divided in half by a partition board (Fig. 3). Soil used for the experiment was andosol brought from the forest floor around the laboratory. Rocks, roots, and litter were removed from the soil by sifting with a sieve of 5 mm mesh. As soon as the cages were set up, the frogs were introduced into them, and the frogs were allowed to burrow into the soil. The cages were placed outside in the shade for 48 hours. Afterwards, the frogs were dug out to observe the effect of soil conditions on burrowing. The temperature of the experimental cages was not controlled, and ranged from -3.3 to 18.5°C.

The effects of soil hardness, soil moisture, and presence of litter in soil layer on frog behavior were examined from December 1997 to February 1998. The experimental period was one week. During this period the frogs were put outside in the plastic containers (40×30×30 cm) which contained 20 cm of damp soil with thin leaf litter.

Based on the results of measurements at the hibernation points, the soil hardness was set at 0.1 kg/cm² and 2.5 kg/cm² using soil with 30% water content.

In experiments about moisture, soil with water content of 30% and 15% (soil hardness of 0.1 kg/cm²) was used. These values were dryness ratios of soil samples from hibernation point P and from the smallest water saturation point of the study points, respectively. Ten g of each sample was oven-dried at 105°C for 24 hours and weighed to obtain the dry ratio.

To test the effect of presence of a litter layer, litter was laid thinly on soil to a depth of 5 mm

on one side of the cage and covered with a soil layer to a depth of 5 mm, and seven fallen leaves were placed on top. On the other side no litter was laid on the soil. The litter used in this experiment originated from *Cinnamomum camphora* which is a close relative of *Cinnamomum japonicum*, the dominant tree species around site A. The shape of leaves of both the species are very similar. In this experiment, soil hardness was 0.1 kg/cm² and soil water content was 30%. Fallen leaves which were dry and unbroken were used in this experiment.

RESULTS

Location of hibernation sites and captured individuals.—A total of 26 frogs hibernating in upper layers of the soil were detected only at two bottom sites in the valleys. The two bottom sites where hibernating individuals were concentrated were located at the edge of ponds and on the banks of streams. Frogs were found neither at the upper part of the hill nor on the slopes of the valleys (Fig. 1). Immediately after hibernating frogs were dug out, they excreted much urine. The body sizes of frogs captured were 20–53 mm SVL ($\bar{x} \pm 1SD = 30.0 \pm 0.9$, $N = 26$). Of these frogs, nine were more than 30 mm in SVL and were regarded as adults (six males and three females). The remaining 17 frogs were regarded as juveniles (sex undetermined). The

depth at which frogs were found ranged from 0.0 to 5.6 cm ($\bar{x} \pm 1SD = 3.1 \pm 1.6$). The frog found at the shallowest position was located just under litter on the soil surface. There was no significant correlation between SVL (body length) and the depth of the hibernating position ($r = 0.20$, $p > 0.1$).

Soil conditions of hibernation points.—The two points where hibernating individuals were concentrated were very limited, each smaller than 6 m² (Fig. 2).

Soil hardness of the hibernation points ranged from 0.1 to 2.1 kg/cm² ($\bar{x} \pm 1SD = 1.0 \pm 0.5$, $N = 25$) at the edge of the pond (P), and from 0.3 to 3.1 kg/cm² ($\bar{x} \pm 1SD = 1.1 \pm 0.7$, $N = 25$) at the stream side (S) (Fig. 4). Soils of the hibernation points were significantly softer than those of the other points (Kruskal-Wallis test, $H = 98.97$, $p < 0.001$).

FIG. 1. Study area. Circles indicate search sites. Black circles show the hibernation sites. The numbers of individuals captured were 17 and nine at sites A and B, respectively.

FIG. 2. Site A. The frame indicates the range of detailed searches for hibernation points. Hibernating individuals were observed in limited areas, at edge of a pond (P) and at the slope on the side of a stream (S). Hibernating individuals were not found at the upper side (PU) or lower side (PL) of a hillside, at the slope at the side of a stream (SO), or at the slope at the lower reaches of a stream (SL).

FIG. 3. Plastic container (35×20×27 cm) for the experiment of soil condition selectivity.

Water saturation percentage of the hibernation points ranged from 83.9 to 85.6% ($\bar{x} \pm 1SD = 84.9 \pm 0.9$, $N=3$) at P, and from 76.6 to 87.7% ($\bar{x} \pm 1SD = 82.5 \pm 5.6$, $N=3$) at S (Fig. 5). These values were similar to those at PL, SO, and SL, and water saturation percentage at PU (34.8–58.7%, $\bar{x} \pm 1SD = 50.4 \pm 13.5$, $N=3$) was significantly lower than at the other points (Kruskal-Wallis test, $H=12.99$, $p<0.05$).

Soil temperature of the hibernation points ranged from 5.3 to 8.2°C ($\bar{x} \pm 1SD = 6.4 \pm 1.3$, $N=6$) at P, and from 5.0 to 7.8°C ($\bar{x} \pm 1SD = 6.1 \pm 1.3$, $N=6$) at S. The average soil temperature at the hibernation points was significantly lower than at the other points without hibernating frogs (Friedman test, $\chi^2 = 13.43$, $P<0.05$). The coefficient of variation (CV %) of soil temperature at the hibernation sites was 19% at P, and 20% at S. These values were similar to those at PL, SO, and SL, but the

FIG. 4. Frequency distribution of soil hardness (kg/cm²). I, <1 kg/cm²; II, 1–1.9 kg/cm²; III, 2–2.9 kg/cm²; IV, >3 kg/cm². Soil hardness was measured in January 1998.

FIG. 5. Water saturation percentage and soil temperature at the six study points. Values of water saturation are the means of three measurements. Standard deviation in error bars. Triangles, circles, and diamonds show highest, mean, and lowest temperatures, respectively.

coefficient of variation of PU (27%) was wider than that of the other sites. It appears that hibernation sites were constantly cooler than the other sites.

The thickness of the A₀ layer of the hibernation points was 2.0–3.0 cm ($\bar{x} \pm 1SD = 2.5 \pm 0.5$, $N=3$) at P, and 1.0–1.5 cm ($\bar{x} \pm 1SD = 1.0 \pm 0.5$, $N=3$) at S, which were a little thicker than at PL and SO. The thickness of the A₀ layer at PU and SL was, however, significantly greater than that at the other points (Kruskal-Wallis test, $H=14.88$, $p<0.05$; Table. 1). The ground surface at PU and SL was covered with a thick litter layer. But the A₀ layer at hibernation point S, at PL, and at SO lacked a litter layer, and the thickness of litter layer at hibernation point P was significantly less than that at PU (t-test, $p=0.003$) and SL (t-test, $p=0.002$).

Burrowing behavior under different soil conditions.—When frogs were allowed to burrow under different soil hardness, all frogs selected the soil of low hardness (binomial test, $p<0.0001$). Larger frogs hid deeper than smaller ones did, as there was a significant correlation between the body length and the position (the depth) of the frog in soil ($r=0.7$, $p<0.05$). In the humidity experiment, eight of nine adults and 15 of 17 juveniles selected the

TABLE 1. Thickness (in cm) of the three layers which compose A₀ layer of the study points. The thickness is shown as a mean ($\pm$ 1SD) of three replicates in each case.

Study point	Litter layer	Fermentation layer	Humus layer	Total (A ₀ layer)
P	0.2 $\pm$ 0.3	0.5 $\pm$ 0.5	1.8 $\pm$ 1.0	2.5 $\pm$ 0.5
S	0.0	0.2 $\pm$ 0.3	0.8 $\pm$ 0.3	1.0 $\pm$ 0.5
PU	1.6 $\pm$ 0.3	1.8 $\pm$ 0.3	2.8 $\pm$ 1.2	6.2 $\pm$ 1.8
PL	0.0	0.0	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2
SO	0.0	0.0	0.2 $\pm$ 0.3	0.2 $\pm$ 0.3
SL	2.7 $\pm$ 0.5	3.2 $\pm$ 0.3	3.5 $\pm$ 0.5	9.3 $\pm$ 1.3

soil of high humidity (binomial test, $p < 0.0001$). In the third experiment to examine the effects of the presence of a litter layer, eight of nine adults burrowed in the soil without a litter layer (binomial test, $p < 0.05$), and 10 of 17 juveniles burrowed in the soil without a litter layer. Juveniles did not show any selectivity with regard to litter layer (binomial test, $p > 0.1$)

DISCUSSION

Hibernating *R. schlegelii* were found in relatively shallow layers of the soil only at the bottom of valleys. The soil conditions of hibernation sites were characterized by the common features of low hardness, high water saturation, lack of thick litter layer, and constantly cool temperature.

The results of these field observations lead to a strong supposition that the frogs behaviorally select particular soil conditions for their hibernation sites. In the experiments regarding soil hardness, all frogs actually selected softer soil to burrow into. Average soil hardness in the field where the frogs were found was 1.0 kg/cm², which was slightly harder than the experimental setting. This may be the reason why the frogs burrowed deeper (4.9 cm) in the laboratory than in the field (3.1 cm). In Aomori Prefecture, *R. schlegelii* were found to hibernate at 10 cm below the soil surface (Wada, 1936). But this hibernating point was frequently plowed as vegetable or rice field so that the soil might be much softer than the points in the present study.

The colder winter in Aomori Prefecture (41°N) compared with the Miura Peninsula (35°N) might also have led the frogs to burrow deeper to avoid freezing. At any rate, the limited burrowing ability of tree frogs certainly needs soft soil to burrow in order to escape either from freezing or desiccation.

In the experiment examining the effect of soil moisture on burrowing, frogs clearly selected the moister soil. In the field, the *R. schlegelii* were

hibernating around streams or ponds in a small valley where the soil was moister than on the hillside. These observations suggest that *R. schlegelii* select moist places for hibernation. Undoubtedly, hibernating in moist soil, which is influenced by underground water, reduces evaporative body water loss and obtains water during dry winters. But the burrowing ability of this frog is clearly limited and extremely less than that of the spadefoot toad *Scaphiopus hammondi*, which can burrow 30–90 cm deep to get water from wet soil (Ruibal et al., 1969). Therefore, *R. schlegelii* select moist places with softer soil to escape from winter dryness and freezing.

In the field, the A₀ layer of hibernation points was lacking in thick litter layers. In the experiments, the juveniles burrowed in soil both with and without a litter layer, but the adults whose ability for burrowing is evidently greater than that of the juveniles were biased towards the absence of a litter layer. The results from the juveniles indicate that the litter layer did not directly obstruct the burrowing of the frogs.

The results of this experiment with the adults could be related to their need for water absorption from the soil. Since terrestrial frogs are known to absorb water from soil through their ventral posterior skin, thus they must keep their ventral side in contact with the soil to absorb water (Duellman and Trueb, 1986; Stebbins and Cohen, 1995). In the experiments, litter layer covering the soil surface could prevent water absorption by the frogs, so the adults had to move to a place without a litter layer. The juveniles, which are smaller than adults, could, however, use the small interspaces of the litter to absorb water from the soil. In the field, the frogs did not hibernate in places with a thick litter layer because it prevents water absorption. These results suggest that *R. schlegelii* chooses suitable moist places for hibernation on the basis of the possibility of water absorption.

Soil temperature of the hibernation sites of *R. schlegelii* was constantly cool. Hisai and Sugawara (1978) suggested that hibernating *Bufo japonicus* determine the timing of awaking by the rise in soil temperature. Thus, it is supposed that a constantly cool site has the advantage of avoidance of temporary changes in soil temperature caused by abrupt rises in air temperature during unsuitable seasons.

ACKNOWLEDGMENTS.—I would like to thank Prof. Junichi Aoki, Associate Professor Dr. Nobuhiro Kaneko, Dr. Masami Hasegawa, and Mr. Mituhiko Toda for their valuable comments and suggestions, and Mr. Kei Ichisawa, graduate student of the Department of Soil Zoology, Yokohama National University, for his assistance in this study.

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