Burrow Use by *Salamandrella keyserlingii* (Caudata: Hynobiidae)

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Burrow Use by *Salamandrella keyserlingii* (Caudata: Hynobiidae)

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We determined the use of refugia by salamanders during daytime in summer and characteristics of these refugia in *Salamandrella keyserlingii* at Shaamar, Mongolia. Refugia were located at mean distance of 4.17 m from the edge of the water. Among these refugia, blind tunnels within grasslands (mean distance = 7.50 m) were farther apart than open burrows distributed along a steep slope of a pond-shore (2.49 m). Subterranean burrows having mean depth of 15.4 cm were utilized temporarily (individuals captured only once) with proportional habitat use of 0.704 (19/27 refugia). Mean temperature was lower in burrows/under logs (16.22°C) than in ambient air (26.70°C) or among grasslands (25.10°C). Mean relative humidity was higher in burrows/under logs (85.54%) or among grasslands (75.53%) than in ambient air (48.33%). Mean illumination intensity was 27.0 lx in burrows/under logs and 17,188.1 lx on the surface out of refugia. Mean soil pH was 7.52 beneath salamanders in refugia.

SMALL mammals such as prairie dogs and rodents use burrows as refugia to decrease predation risk (Hoogland, 1995; Ebensperger and Blumstein, 2006). Avian species such as parrots and trogons also use burrows as nest-sites to avoid predators (Brightsmith, 2005). By contrast, in a case of a species with little predation risk, male and female desert tortoises co-occupy burrows to court and mate (Bulova, 1994). In amphibians, burrows may function to reduce desiccation as well as predation (Kolbe et al., 2002; Rothermel and Luhring, 2005).

The majority of amphibian species have complex life cycles, alternating between aquatic and terrestrial phases. In ambystomatid, hynobiid, and salamandrid salamanders, postbreeding adults ''emigrate'' (but do not disperse: Semlitsch, 2008) upland, find refuge in subterranean burrows or under cover objects such as decaying logs, woody debris, rotting leaves, moss mats, and stones, and cannot be readily found outside of the breeding season (Duellman and Trueb, 1986). As a result, the ecology of these salamanders during the nonbreeding season is poorly understood (Verrell and Davis, 2003; Hasumi and Kanda, 2007). Although ambystomatids have been well documented to occupy small mammal burrows (Kolbe et al., 2002; Rothermel and Luhring, 2005), burrow use by hynobiid salamanders has not been confirmed and anecdotal evidence is also lacking (Kusano and Miyashita, 1984).

Salamandrella keyserlingii has the broadest range of any amphibian species worldwide (Duellman and Trueb, 1986). The species range covers Siberia from eastern Europe to Kamchatka and occurs sporadically in China, Japan, and Mongolia (Borkin, 1999). Within Mongolia the occurrence of *S. keyserlingii* is known at Shaamar (Borkin and Kuzmin, 1988), but its microhabitat use has not been documented. In a preliminary survey, we discovered a salamander inhabiting a burrow. The aim of our study was to determine whether burrow use by salamanders was ''temporary use'' or ''repeated or continual use'' (Madison and Farrand, 1998) and to determine whether these microhabitats were favorable or unfavorable as refugia based on measurements of physical parameters such as temperature, relative humidity, illumination intensity, and pH (Sugalski and Claussen, 1997).

MATERIALS AND METHODS

Study area.—We conducted this study at Shaamar, Selenge Province, Mongolia, within 25 km of the border between Mongolia and Russian Federation (50°04′N, 106°07′E; 600 m elevation; sunset occurred at 2130–2200 h during mid-July). The site was a wetland complex containing several ponds, sedge meadows, and discontinuous grasslands in floodplains of the Selenge and Orkhon Rivers. Unlike another Mongolian location for this species, Darhadyn Wetland (Hasumi et al., 2007), there were few decaying logs on land and hence a lack of cover objects.

Salamandrella keyserlingii is nocturnal in both the spring (Hasumi and Kanda, 2007) and summer (Grigoriev and Erdakov, 1981). Salamanders forage above ground at night and hide in cool, moist refugia during daytime. Available refugia were subterranean burrows located in two settings: along a steep slope (60–80°) of a pond-shore, without much grassland vegetation (open burrows); and within grasslands (blind tunnels). Muskrats (*Ondatra zibethicus*) were presumed to construct the open burrows because six individuals were trapped in nylon mesh traps set near the edge of the pond-shore (M. Hasumi, unpubl. data). There was no evidence that salamanders excavated the blind tunnels (Madison and Farrand, 1998). There were no burrows around five other ponds examined, where we could not find any salamanders. The grasslands located between the six ponds examined (pond–pond distance from 80–500 m) could be a movement barrier for salamanders (Rittenhouse and Semlitsch, 2006). Judging from this preliminary survey, movement of *S. keyserlingii* out of the study area was unlikely (i.e., absence of metapopulation: Kolbe et al., 2002; Smith and Green, 2005).

Monitoring techniques.—We selected one of the six ponds examined on 16 July 2005 and mapped the surrounding region. Because livestock frequently used one side of the pond, we examined a 0.5-ha area on the opposite side of the pond and divided the area into three subareas, to concentrate limited persons in a small area: area 1 (46.8 × 30 m), area 2 (62.6 × 30 m), and area 3 (63.6 × 30 m).

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We conducted daily surveys from 17–24 July 2005 (Day 1–Day 8). Six observers searched for salamanders in subterranean burrows, under decaying logs, and among grasslands in area 1 (for 2.0 h), area 2 (2.5 h), and area 3 (1.5 h). We based time-constraints on the number of potentially appropriate refugia. Immediately after finding an individual, we recorded the time and temporarily kept the salamander in a cooler box with moist sphagnum moss to prevent desiccation. We assigned each refugium a number and plotted its location on a map. We measured, using a measuring tape, size of each refugium (depth of burrows as estimated by inserting either finger or arm, or length and diameter of logs) and distance between the refugium and the edge of the water. We determined the following physical parameters: temperature and relative humidity of the ambient air 1.5 m above each refugium and, when appropriate, inside refugium using a thermo-hygrometer; illumination intensity inside and outside of each refugium with a light meter; and soil pH beneath each individual in a refugium with a compact, portable pH-meter (calibrated immediately before measurement with pH 7 and pH 4 standard solutions). We placed the soil in a sensor box of the pH-meter and then immersed it with distilled water before measuring pH. We regarded 19,990 lx or more illumination intensity as ''19,990 lx.''

We recorded age class, sex, and visual characteristics such as throat coloration and dorsal color pattern of each individual, according to Hasumi (2001). We weighed each individual (body mass: BM, to within 0.05 g) and measured the broadest head width (HW), maximum tail height (TH), snout–anterior vent length (SAVL: distance from the tip of the snout to the anterior angle of the vent), snout–posterior vent length (SPVL: from the tip of the snout to the posterior angle of the vent), and tail length (TL: from the posterior angle of the vent to the tip of the tail) to within 0.01 mm by a modification of Wise and Buchanan's (1992) method without using anesthesia. Based on these measurements, we categorized all individuals into five classes: adult males, adult females, unsexed individuals, juveniles, and metamorphs or individuals that completed metamorphosis within the last month. Adult females were identified as those with a beige ovisac, visible through the skin of the ventral region of the torso near the hindlimbs despite earlier reports that ovisacs were visible only in fall–spring (Hasumi, 1996). Because of difficulty in distinguishing metamorphs from juveniles by body size, we used skeletochronological results for the distinction (see below).

We marked salamanders individually using up to one toe clip per appendage and fixed the clipped toes in 10% neutral buffered formalin *in situ*. We released marked salamanders to the refugium of capture, after which we attempted to reconstruct it. If a recapture was found in a new refugium, we determined distance between these refugia with a measuring tape. We did not examine body size characteristics of recaptured salamanders.

Skeletochronology.—We conducted all skeletochronological procedures according to Hasumi and Watanabe (2007). We counted the number of lines of arrested growth (LAG) in each specimen based on the premise that the number of LAGs does not differ between femurs, humeri, and phalanges. We estimated age of each individual as

$$a + (b - c)/365,$$

where ''a'' = number of LAGs, ''b'' = Julian date of capture, and ''c'' = Julian date of estimated completion of breeding (1 June = 152). We did not fit any nonlinear growth equation between age and body size because of small sample size (n = 23).

Statistical analysis.—We compared mean temperature, relative humidity, or illumination intensity between refugia, mean BM, SPVL, or age between sexes, and mean distance from the edge of the water between burrow types, using a Student's *t*-test for equal variances or the Aspin-Welch test when exhibiting unequal variances.

RESULTS

Overview of captures.—We captured 27 individuals, each around a different refugium. The number captured per day ranged from 1–5. Out of those 27 individuals, seven males, four females, and eight juveniles were found in 19 burrows; two males, one female, and two juveniles, under five logs; and one male and two metamorphs, among three grasslands. On most occasions, we had access to individuals at the bottom of burrows. We marked 23 individuals (nine males, four females, eight juveniles, and two metamorphs) and recaptured four (one male, one female, and two juveniles). A male moved 19.6 m from a log (Day 1) to a burrow (Day 4). A female and a juvenile moved among burrows with 6.1 m and 3.0 m distances, respectively, for four days (Day 1–Day 5). A juvenile moved 13.6 m from a burrow (Day 1) to a log (Day 5). Mean movement distance of individuals per day was 3.05 m (n = 4, range = 0.75–6.53, SD = 2.57).

Refugium characteristics.—Proportional use of each refugium by salamanders was 0.704 for burrows (n = 19), 0.185 for logs (n = 5), and 0.111 for grasslands (n = 3). Refugia were located at mean distance of 4.17 m from the edge of the water (n = 27, range = 0.7–9.9, SD = 3.02). Among those 19 burrows, blind tunnels were located within the grasslands at mean distance of 7.50 m from the edge of the water (n = 7, range = 0.8–9.9, SD = 3.38). These tunnels were farther apart than open burrows (t = 3.687, df = 8, P = 0.0061), distributed along the steep slope of the pond-shore, at mean distance of 2.49 m (n = 12, range = 0.7–6.6, SD = 1.58). Mean burrow depth was 15.4 cm (n = 19, range = 7–39, SD = 8.5). Mean length of log refuges was 64.0 cm (n = 5, range = 20–115, SD = 41.4), log diameter was 10.6 cm (range = 8–18, SD = 4.2), and log area (length × diameter) was 659.0 cm^2 (range = 160–1000, SD = 390.4).

We found no detectable difference in physical parameters between burrows and logs, and thus data on these two refugia were combined for the following analyses (Table 1). Mean temperature was lower in burrows/under logs (16.2°C) than in ambient air (26.7°C) or among grasslands (25.1°C). Mean relative humidity was higher in burrows/under logs (85.5%) or among grasslands (75.5%) than in ambient air (48.3%). Both temperature and relative humidity tended to be high among grasslands. Mean illumination intensity was lower in burrows/under logs (27 lx) than on the surface (17,188 lx). Mean soil pH was 7.52 beneath salamanders in refugia (n = 27, range = 5.3–8.9, SD = 1.00). There was no significant difference in temperature (P = 0.1938), relative humidity (P = 0.5000), or illumination intensity (P = 0.3288) between blind tunnels and open burrows.

Table 1. Mean Measurements ± 1 SD (Range) of Temperature, Relative Humidity, and Illumination Intensity in Outside/Open (n = 27), Burrow/Log (n = 24), and Grassland (n = 3) and Statistical Comparisons of Each Parameter between Sites (Asterisk: NS at α = 0.05).

Parameter	Outside/open (O)	Burrow/log (B)	Grassland (G)		t	df	P
Temperature	26.70 ± 2.59	16.22 ± 3.33	25.10 ± 4.81	O vs. B	12.615	49	<0.0001
(°C)	(20.1–31.2)	(9.1–23.1)	(19.6–28.5)	O vs. G	0.567	2	0.6276*
				B vs. G	4.173	25	0.0003
Humidity	48.33 ± 9.44	85.54 ± 4.33	75.53 ± 8.63	O vs. B	18.406	37	<0.0001
(%)	(22.9–72.0)	(76.2–92.7)	(69.4–85.4)	O vs. G	4.760	28	<0.0001
				B vs. G	1.978	2	0.1865*
Illumination	17,188.1 ± 3999.1	27.0 ± 52.5	6716.7 ± 11,495.1	O vs. B	22.296	26	<0.0001
(lx)	(7820–19,990)	(1–207)	(31–19,990)	O vs. G	1.567	2	0.2576*
				B vs. G	1.008	2	0.4196*

Body size and age.—BM, SPVL, and age are shown in Table 2 for each sex or age class, but other parameters (HW, TH, SAVL, and TL) were not shown. Males and females did not differ significantly in BM (P = 0.3109), SPVL (P = 0.8191), and age (P = 0.1164). Minimum age at maturity–maximum longevity were 5.14–9.13 years for males and 7.13–9.14 years for females. Juveniles did not include any 1–2 year-old young.

DISCUSSION

Refuge use by salamanders differed at site, population, or species. Burrow use at Shaamar was temporary (individuals captured only once, eight-day surveys) and individuals were scattered (never collected together). In contrast, at Darhadyn Wetland the same salamanders were located in the same common log refuges (M. Hasumi, unpubl. data). For example, even in a shorter temporal scale in 2004 (four-day surveys) one individual was captured from the same log four times (every day), and in 2005 (ten-day surveys) two individuals were captured from each of the same logs eight times. In *Ambystoma tigrinum*, repeated or continual use of subterranean burrows by one or more salamanders was suggested (but mark–recapture studies were not conducted: Kolbe et al., 2002), and salamanders excavated tunnels for temporary refuge but used small-mammal runway systems for long-term refuge (Madison and Farrand, 1998). However, as stated previously, there was no evidence for the excavation of blind tunnels in *S. keyserlingii*.

Our small sample size (n = 27) may be inadequate for the conclusion of whether the lack of repeated use of a refuge site indicates that refuges are temporary. We do not think our burrow disturbance biased measurements of salamander burrow use. This is likely both because salamanders were recaptured from four disturbed refugia (three burrows and one log) and because the similar log disturbance did not have any bias against log use continuity at Darhadyn Wetland (M. Hasumi, unpubl. data). In this wetland, resident individuals remained under the same logs and shared refugia with other individuals (maximum sharing number per day = 8 individuals). Yet, concern still remains about the effect that investigations had on the integrity of the burrows, perhaps causing the salamanders to abandon their refuges for other sites (i.e., researcher-mediated effect on refugia).

Hoogland (1995) recorded mean relative humidity of 88% in burrows. In summer, access to burrows would allow salamanders to remain underground during daytime and to become active above ground at night, which decreases a risk of predation and desiccation (Kolbe et al., 2002). In *Ambystoma talpoideum*, 90% of salamanders with access to a burrow survived, whereas only 40% survived without burrows (Rothermel and Luhring, 2005). Climatic conditions at our study area, such as low relative humidity (mean = 48.3%, range = 22.9–72.0) and high temperature (mean = 26.7°C, range = 20.1–31.2) during the day, may be physiologically stressful to nocturnal amphibians. In contrast, in *S. keyserlingii* daytime burrows there were high relative humidity (in burrows/under logs: mean = 85.5%, range = 76.2–92.7) and low temperature (mean = 16.2°C, range = 9.1–23.1). These daytime burrows therefore functioned to reduce desiccation and heat, as well as predation, for a favorable refuge of salamanders.

Ambystomatid salamanders spend most of their life in inaccessible underground retreats (subterranean burrows) and rarely move above ground (Regosin et al., 2004). Although it is not known whether the majority of hynobiids utilize subterranean burrows, we found that *S. keyserlingii* inhabited muskrat burrows at Shaamar. Burrow depths used

Table 2. Measurements of Body Mass (BM), Body Size (Snout–Posterior Vent Length: SPVL), and Age, Expressed by Mean ± 1 SD (Range), in Each Sex or Age Class. A distinction of 0-year metamorphs from multi-year juveniles was based on skeletochronological results.

Class	n	BM (g)	SPVL (mm)	Age (years)
Male	9	5.99 ± 1.37 (3.35−7.80)	62.28 ± 5.09 (51.80−68.17)	7.24 ± 1.16 (5.14−9.13)
Female	4	5.14 ± 1.27 (3.50−6.55)	61.53 ± 5.92 (54.35−66.82)	8.38 ± 0.96 (7.13−9.14)
Juvenile	8	2.12 ± 0.39 (1.50−2.60)	44.53 ± 3.27 (40.47−48.96)	4.38 ± 0.88 (3.13−5.14)
Metamorph	2	0.65 (0.60−0.70)	29.43 (28.30−30.56)	0.14 (0.14)

by 19 individuals ranged from 7–39 cm, and many were limited to the steep slope of the pond-shore, similar to *A. tigrinum* (Madison and Farrand, 1998), resident salamanders of which remained close to a breeding pond. In comparison, 18 of 25 *Triturus carnifex* emigrants were discovered using rodent burrows that were 5–80 cm in depths under tree roots (Schabetsberger et al., 2004). Nonmigratory surface activity (aboveground movement) was suggested for *Hynobius tokyoensis* (Kusano and Miyashita, 1984), but this suggestion was based on finding salamanders at the entrance of burrows or under cover objects above ground. Well-known burrow use by ambystomatids seems to have led to the assumption that the majority of hynobiids also utilize subterranean burrows. However, since burrow use *per se* was not verified in *H. tokyoensis*, our documentation on *S. keyserlingii* is the first to report burrow use by hynobiids.

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