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Role of Substrate Cues in Habitat Selection by Recently Metamorphosed *Bufo terrestris* and *Scaphiopus holbrookii*

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Abstract.—Amphibians exhibit high rates of evaporative water loss that can affect their distribution, movements, and patterns of habitat use. Forest clearcutting alters habitat and results in environmental changes such as canopy removal and leaf litter loss that may promote drier microclimates in harvested clearcuts. Subsequently, clearcutting has been shown to generally reduce amphibian abundances and richness. We investigated the role of substrate cues in habitat differentiation between clearcuts and forests in juvenile Southern Toads (*Bufo terrestris*) and Eastern Spadefoot Toads (*Scaphiopus holbrookii*) in laboratory experiments. Neither *B. terrestris* nor *S. holbrookii* exhibited a preference for a single substrate when offered the choice between forest soil and clearcut soil. However, *S. holbrookii* significantly preferred forest substrate over clearcut substrate when forest litter was added to the forest soil. The affinity for forest litter exhibited by juvenile *S. holbrookii* suggests that the availability of suitable microhabitats may be an important determinant of *S. holbrookii* distributions and may explain previously reported habitat associations in field studies.

The loss of suitable habitat has been implicated in the decline of many amphibian populations (Alford and Richards, 1999; Stuart et al., 2004). Even-aged forest management, with associated practices such as clearcutting and extensive site preparation, is one form of land use that has been shown to negatively affect amphibians (e.g., Enge and Marion, 1986; Petranka et al., 1994; Ash, 1997). Because amphibians often exhibit high rates of evaporative water loss (Spight, 1968; Spotila and Berman, 1976) with low cutaneous resistance to water flux (Adolph, 1933; Young et al., 2006), environmental conditions such as temperature and water availability affect their activity (Kam and Chen, 2000), distributions (Gibbs, 1998), and migratory movements (Todd and Winne, 2006). Clearcutting removes canopy cover and reduces soil litter and associated ground cover (deMaynadier and Hunter, 1995; Russell et al., 2004), changing the distribution and availability of suitable microhabitats that amphibians may use to avoid desiccation or to evade predators.

Southern Toads (*Bufo terrestris*) and Eastern Spadefoot Toads (*Scaphiopus holbrookii*) are common throughout most of the southeastern United States. As anurans, they are more tolerant to body water loss than are many salamander species (Thorson and Svihla, 1943; Hillyard, 1999). *Bufo terrestris* have been documented in clearcuts (Ryan et al., 2002; Todd and Rothermel, 2006), and they frequently appear in early successional or altered habitats (Jensen, 2005). In contrast, *S. holbrookii* are much more abundant in forests than in adjacent clearcuts (Enge and Marion, 1986). The identification and preference of one type of substrate by toads may partially explain previously observed abundance patterns. Because recently metamorphosed anurans are smaller and have comparatively greater surface area to volume ratios than adults, they lose water more quickly in proportion to their size. Thus, recently metamorphosed *B. terrestris* and *S. holbrookii* may be more likely than adults to rely on substrate cues to locate forest habitat if desiccation is an important factor, making them useful subjects for understanding the role of substrate cues in habitat selection.

We conducted a series of laboratory experiments to examine whether recently metamorphosed *B. terrestris* and *S. holbrookii* use substrate cues to select between clearcut and forest habitats. Based on published field studies of habitat associations in adult and juvenile animals, we predicted that recently metamorphosed *B. terrestris* would not significantly favor forest substrates, whereas juvenile *S. holbrookii* would.

Materials and Methods

Animal Collection.—We collected recently metamorphosed *B. terrestris* and *S. holbrookii* from the Savannah River Site (for further site description, see Gibbons et al., 1997) in Aiken and Barnwell County, South Carolina, from 6 June 2005 to 18 June 2005. We collected *S. holbrookii* as they metamorphosed and emigrated from a seasonal wetland, Bay 5148, and we collected *B. terrestris* as they metamorphosed and emigrated from a semipermanent wetland, Ellenton Bay. We collected all animals by hand in the morning (0600–1000 h) while they were active. We maintained all animals with 14 h of light at cool temperatures in plastic containers with paper towels moistened with aged well water. We used the animals in laboratory experiments within 1–4 days of collection.

Soil Experiments.—We collected all soil used in the experiments from the upper 3 cm of a pine forest and from the upper 3 cm of an adjacent, recently clearcut pine forest (1 yr old; harvested in April 2004). We sifted all soil through a 6-mm sieve to remove debris and minimize textural differences in the soil. Because amphibians often prefer moist soil to dry soil (e.g., Rittenhouse et al., 2004), the sifted soils were spread separately on plastic sheets and allowed to dry completely for 24 h to eliminate moisture differences before being used in choice tests. Ten 38-liter glass aquaria were used in all choice tests. The dimensions of the aquaria were 50 cm length × 27 cm width ×

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30.5 cm height. We placed dry clearcut soil to a depth of 5 cm on one side of each aquarium and dry forest soil to a depth of 5 cm on the other side in a systematic arrangement so that the side containing forest soil alternated from one aquarium to the next. The soils met along the middle and were kept from mixing with a thin divider buried in the soil. Half of the aquaria were arranged north-south and the other half were arranged east-west to avoid biases that may have resulted from directional orientation in the juvenile animals. Each glass aquarium had its sides wrapped in dark paper to prevent animals from observing the activity of conspecifics in other aquaria. Prior to commencement of the experiments, both halves of the aquaria were moistened with equal amounts of deionized water from a spray bottle, resulting in wet, but not saturated, soils. A 5-cm diameter piece of quarter-inch screen was used as the release platform at the center of each aquarium. This prevented the toads from digging into the soil before release while allowing them to receive cues from the soils. We allowed animals to acclimate on the screen under a small opaque release container for 10 min before commencing the experiments. All trials took place on consecutive days in a temperature-controlled room maintained at 24°C under low light in the early morning (0600 h), approximating capture conditions. Forty animals (20 *B. terrestris* and 20 *S. holbrookii*) were used in the experiment, and 10 individuals of one species were used alternately for four days. Soils were changed and aquaria were bleached and rinsed thoroughly between each trial. We observed the animals from behind a blind and recorded their initial soil preferences after gently removing the covers of their release chambers and again after 5 min. Finally, we recorded their locations at 2 h and 4 h postrelease. Animals that were found at the centerline junction of the two soils were recorded as exhibiting no preference.

Combined Substrate Experiments.—Combined substrate tests were conducted in the same manner as the soil tests above, using 40 new animals (20 *B. terrestris* and 20 *S. holbrookii*). However, approximately 5 cm of dry pine litter was added to the sides of the aquaria that contained forest soil. Pine litter was collected from the same location from which the forest soil was collected. A few dry leaves and weathered pine needles that were collected from the clearcut were added to the sides of the aquaria containing clearcut soil. Thus, the halves of the aquaria containing clearcut soil had approximately 2 cm of decayed litter with significant patches of bare soil, closely approximating the condition of clearcuts on the Savannah River Site (Rothermel and Luhring, 2005). All litter was dried similarly to the soils. Both soils were moistened equally with deionized water from a spray bottle as described above, prior to adding litter and commencing the trials.

Statistical Analyses.—To determine whether toads selected one substrate more than expected at random, we used a two-tailed binomial test at four time intervals: initial release, 5 min, 2 h, and 4 h after release from the holding chamber. Animals found in the center of the enclosures were recorded as not exhibiting a choice and were excluded from analysis for that time interval. Significance was determined in all cases at the $\alpha = 0.05$ level, and data were analyzed using SAS® version 9 (SAS Institute, Inc., Cary, NC, 2000).

Results

Bufo terrestris and *S. holbrookii* did not significantly favor either soil at any of the four observation periods in the soil tests (Table 1). At the conclusion of the soil tests, 14 of 20 *S. holbrookii* had burrowed into substrate, whereas none of the *B. terrestris* had. In the combined litter-soil tests, *S. holbrookii* selected the combined forest litter and soil substrate more frequently than the clearcut substrate at 2 h and 4 h ($P \leq 0.01$) but did not exhibit an initial preference or a preference at 5 min (Table 1). All 20 *S. holbrookii* had burrowed into substrate by the conclusion of combined substrate tests. *Bufo terrestris* did not significantly favor either combined substrate at any observation period (Table 1), and none of them had burrowed into the substrate at the conclusion of the experiment.

Discussion

The results of the current study agree with published observations of habitat associations with juvenile and adult *B. terrestris* and *S. holbrookii*. Specifically, adult *B. terrestris* have been reported in clearcuts (Ryan et al., 2002), whereas adult *S. holbrookii* have been shown to be more abundant in forested landscapes (Enge and Marion, 1986). A previous study has also demonstrated that juvenile *B. terrestris* will enter clearcuts as they disperse away from natal wetlands into terrestrial habitat, despite having higher mortality and lower growth rates in clearcuts (Todd and Rothermel, 2006). In contrast, juvenile *S. holbrookii* have not been documented emigrating into clearcuts. In the current study, recently metamorphosed toads of both species did not preferentially select a soil type significantly more than expected at random. However, *S. holbrookii* showed an affinity for forest substrate when representative pine litter was combined with forest soil. *Scaphiopus holbrookii* may prefer forest litter because they associate dense litter with their preferred forest habitats.

Because forest litter may buffer changes in soil moisture and reduce predation and desiccation risks to fossorial amphibians, dense forest litter has been suggested as an important characteristic for amphibians in forested landscapes (deMaynadier and Hunter, 1995; Russell et al., 2004). Indeed, when presented with pine litter in the second substrate choice experiment, most *S. holbrookii* buried into the forest soil beneath the pine forest litter. Thus, although *S. holbrookii* do not appear to be strongly attracted to forest soil in the absence of other habitat cues, they do favor deep forest litter, which may aid them in avoiding desiccation. Preference for forest litter by *S. holbrookii* may also be a product of behavioral predator avoidance, a factor shown to influence substrate selection by recently metamorphosed American Toads, *Bufo americanus* (Heinen, 1993). Additional research on habitat selection may reveal proximate mechanisms used by amphibians to identify suitable habitat, furthering our understanding of the processes that underlie changes to patterns of species abundance and richness in altered habitats.

TABLE 1. Sample sizes and results of two-tailed binomial tests for each of the four intervals in the two different substrate selection experiments.

Experiment	Species	Interval	Number selecting forest/total number making selection	*P*-value
Soil	*S. holbrookii*	initial	6/13	1.00
		5 min	7/20	0.26
		2 h	8/20	0.50
		4 h	8/19	0.65
	B. terrestris	initial	7/15	1.00
		5 min	11/20	0.82
		2 h	6/20	0.12
		4 h	8/18	0.82
Litter and soil combined	*S. holbrookii*	initial	8/10	0.11
		5 min	13/20	0.26
		2 h	16/20	0.01 *
		4 h	17/20	< 0.01 *
	B. terrestris	initial	13/20	0.26
		5 min	13/20	0.26
		2 h	8/17	1.00
		4 h	11/19	0.65

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