

## Ecology of the Pitviper, *Bothrops moojeni*, in the Brazilian Cerrado

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**ABSTRACT.**—*Bothrops moojeni* is a member of the *atrox* group that occurs in central and southeastern Brazil and adjacent Paraguay and Argentina. We describe habitat use, diel and seasonal activity, biometry, feeding habits, and reproduction of *B. moojeni*, based on field studies and analysis of 207 preserved specimens. *Bothrops moojeni* predominantly uses riparian vegetation in the Cerrado (central Brazilian savannas), such as gallery forests and adjacent wet grasslands, although they occasionally use drier interfluvial areas. Sexes are dimorphic in snout–vent length, relative tail length, relative mass, and relative head length. Main prey types were mammals, frogs, and lizards. There is an ontogenetic dietary shift from ectotherms to endotherms. Prey–predator mass ratio ranged from 0.008–1.079, and prey mass was positively correlated with predator mass. Although prey absolute mass was positively correlated with snake mass, large snakes did not eliminate small prey from their diets. There was an ontogenetic decrease in prey relative mass. Females consumed more endothermic prey than males, and this difference probably reflects size differences. The reproductive cycle is lengthy and seasonal, with recruitment restricted to the rainy season. Fecundity is positively correlated with female body size. Life-history characters of *B. moojeni* are similar to those of other members of the *atrox* group, even though *B. moojeni* occurs in the Cerrado, a drier and more seasonal biome.

*Bothrops moojeni* is a large pitviper from riparian areas in central and southeastern Brazil, throughout the Cerrado morphoclimatic domain (Borges and Araújo, 1998; Campbell and Lamar, 1989; Leloup, 1984). Recent systematic studies indicate that populations of *B. moojeni* from the Cerrado of southeastern and central Brazil form a monophyletic assemblage within the *atrox* species group (Wüster et al., 1996, 1999).

Few studies deal specifically with the ecology of snakes in the genus *Bothrops* (Martins and Gordo, 1993; Martins et al., 2002; Sazima, 1992; Valdujo et al., 2002). Regarding *B. moojeni*, basic information on diet, reproduction, and micro-habitat use, directed at rearing for commercial venom production, is available in Leloup (1975, 1984). Borges and Araújo (1998) studied habitat segregation between *B. moojeni* and *Bothrops neuwiedi* in the Brasília region, central Brazil, concluding that the former inhabits mostly gallery forests and adjacent areas, whereas the latter occurs exclusively in open savannas. Martins et al. (2001) reported arboreal habits in juvenile *B. moojeni*, and Martins et al. (2002) reported *B. moojeni* as occurring in gallery forests and swamps in the Cerrado.

Studies on Cerrado fauna are important given the fast rate of destruction of its natural landscapes during the last decades (Ratter et al.,

1997). We present information on habitat use, activity, diet, biometry, and reproduction of *B. moojeni* from central and southeastern Brazil. Preliminary data on the biology of *B. moojeni*, based partially on the same dataset used herein, were provided by Martins et al. (2001, 2002).

### MATERIALS AND METHODS

We obtained field data at several Cerrado localities throughout the range of nominal populations of *B. moojeni* (cf. Wüster et al., 1996, 1999). All study sites were protected areas in undisturbed Cerrado, encompassing all the typical Cerrado vegetation types (see Ratter et al., 1997). We made most field observations and collections at Itirapina Ecological Station (IES, with approximately 2300 ha; 22°15'S; 47°49'W; elevation 800 m), São Paulo, southeastern Brazil; at Emas National Park (ENP, with approximately 130,000 ha; 18°15'S; 52°53'W; elevation 800 m), in southwestern Goiás, central Brazil; and at reserves in the Brasília region, central Brazil, including Águas Emendadas Ecological Station (AEES, with approximately 10,000 ha; 15°33'S; 47°34'W; elevation 1100 m), Área Alfa Cerrado Reserve (AACR, a Brazilian Navy training area with approximately 6,000 ha; 16°00'S; 47°56'W; elevation 1150 m), and IBGE Biological Reserve (IBGE, with approximately 1,350 ha; 15°57'S, 47°53'W; elevation 1100 m). We also made occasional observations in other Cerrado areas in central Brazil, including the regions of

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Chapada dos Veadeiros National Park (about 14°13'S; 47°29'W; elevation 500–1600 m) and Grande Sertão Veredas National Park (about 15°23'S and 45°54'W; elevation 700–1000 m). Cerrado areas in Brazil are characterized by well-defined dry and wet seasons (April to September and October to March, respectively; Eiten, 1972), with annual precipitation around 1500 mm.

Vegetation types in the study areas were divided into two major categories: riparian and interfluvial areas (adapted from Eiten, 1972). Riparian areas consist mostly of gallery forests, wet grasslands (*campo úmido*), and palm marshes (*veredas*) in depressions closely associated with perennial water. Interfluvial areas are covered by several types of seasonally dry savannas, always on higher, gently sloping, well-drained terrains, ranging from grasslands (*campo limpo*) to arboreal savannas (*cerrado sensu stricto*; see Ratter et al., 1997).

We obtained snakes during time-constrained searches (TCS) and incidental sightings (Martins and Oliveira, 1999). We conducted TCS only at the main study sites (IES, Brasília region and ENP), from July 1997 to April 2001. We calculated sampling effort in TCS as the number of person-hours (p-h) spent searching (see Valdujo et al., 2002). For each specimen observed in the field we recorded: time and date of encounter, vegetation and substrate types, posture (see Oliveira and Martins, 2002), and snout–vent length (SVL). We also obtained snakes from local collectors in and around the main study sites; these collectors provided information on habitat use, time, and date of capture for most specimens.

At IES we also obtained snakes from pitfall traps with drift fences (PTDF Greenberg et al., 1994) installed in riparian (three sampling units with 24 buckets) and interfluvial areas (six sampling units with 48 buckets). We used these traps from August 1999 to April 2001. Each sampling unit of PTDF consisted of a pair of 45 m arrays, 100 m from each other. Each array had four 100-liter plastic buckets (placed every 15 m), connected by a 0.5-m high fence made of plastic mesh. Each day of PTDF sampling corresponded to 72 bucket-days. We sampled a total of 185 nonconsecutive days (13,320 bucket-days) from August 1999 to April 2001. Of these, we sampled 124 days (8928 bucket-days) during the wet season and 61 days (4392 bucket-days) during the dry season. We sampled 4440 bucket-days in riparian habitats (border of the gallery forests), and 8880 bucket-days in interfluvial habitats during the course of both seasons. At IES we implanted pit-tags (Trovan® ID 100 Implantable Transponder) in all specimens caught.

To detect eventual differences in seasonal activity using data from local collectors, we compared observed with expected monthly number

of collected snakes, after correcting for unequal monthly sampling efforts (see Seigel, 1992). We considered sampling effort the total number of days of each month, assuming that the probability of finding snakes by local collectors is the same every day. We calculated the monthly expected number of snakes by dividing the total number of snakes collected by the total number of days throughout the study (from April 1997 to April 1999), and then multiplying this value by the number of days of each month. We then compared the observed with the expected number of snakes caught during dry and wet seasons with a chi-square test. To avoid bias caused by juvenile recruitment, we excluded juvenile snakes (SVL ≤ 300 mm) from this analysis.

To obtain data on diet and reproduction, we analyzed 207 preserved specimens from various localities in the Brazilian Cerrado. We deposited all 143 specimens collected for this study in herpetological collections, except for a few in bad state of preservation (e.g., old roadkills or accidentally mutilated specimens). We obtained additional material from the herpetological collections of Instituto Butantan (IB), Universidade de Brasília (CHUNB), and Museu Nacional (MNRJ). We used only museum specimens incorporated in collections without having been previously kept in captivity for long periods.

For each preserved specimen, we recorded the following data (see Shine, 1977a,b, 1986; Shine and Charles, 1982): (1) snout–vent length (SVL); (2) head length (HL); (3) tail length (TL); (iv) body mass after draining the excess of preservative liquid through ventral incisions and removing prey and/or embryos or enlarged follicles (Martins et al., 2001); (5) prey items in gut; (6) sex; (7) sexual maturity (see criteria in Shine, 1977a,b); (8) number of ova or embryos; and (9) diameter of largest ovarian follicle, oviductal egg or encapsulated embryo. All linear measurements were recorded to the nearest millimeter and mass to the nearest gram.

We used combined information from food items found in the stomach and in the hindgut (Martins and Gordo, 1993). The mass of partially digested prey was inferred by comparison with reference specimens of similar size (Greene, 1989). Direction of ingestion was recorded whenever possible. We also recorded tail-tip color, an indicator of caudal-luring behavior (see Andrade et al., 1996; Sazima, 1991). We divided snakes into mature males, mature females, and juveniles for most analyses. In mark-recapture studies at IES, we considered as sexually mature those snakes larger than the smallest mature museum specimen of the same sex.

We tested for sexual size dimorphism in SVL of mature specimens using a Mann-Whitney *U*-test. We tested sexual dimorphism in head length

between mature males and females with an ANCOVA, using trunk length (SVL minus HL) as the covariate and log-transformed variables. We applied the same procedure for testing sexual dimorphism in tail length (SVL as the covariate) and mass (total length as the covariate). Log-transformed variables were used as they reduce heteroscedasticity and promote linear relationships (Zar, 1984; King, 2000).

We explored the effect of snake size on prey size through linear regression analysis. We used the mass of both predator and prey as an indicator of size; both variables were log-transformed. We tested for ontogenetic shift in relative prey size using the slope of the previous regression line, using a *t*-test (see Zar, 1984). We tested for ontogenetic shift in prey type (ectotherms and endotherms) by comparing the frequency of prey types in juveniles and adults with a Fisher exact test. We used the same test to explore sexual differences in diet by comparing the frequency of prey types in mature males and females. We tested for sexual differences in relative prey mass by comparing the prey-predator mass ratios with a Mann-Whitney *U*-test.

We explored the effect of female size on litter size through linear regression analysis, with both variables log-transformed (King, 2000). We performed all statistical analyses using Statistica (Statistica for Windows, Release 5.1 J, Statsoft, Inc., Tulsa, OK, 1998), according to procedures in Zar (1984), with significance levels set at  $\alpha = 0.05$ .

## RESULTS

*Habitat Use and Activity.*—We gathered a total of 69 field records of *B. moojeni*: 26 records during TCS (total of 1205.6 p-h; including four recaptures) in all study areas, 36 records from incidental sightings or interviews with local collectors in all areas, plus seven records from pit-fall traps at IES. Most snakes (92%) found during TCS were in riparian areas, where the encounter rate was 0.051 snake/p-h (22 snakes found during 461 p-h of search). By contrast, in interfluvial savannas, the encounter rate during TCS was 0.005 snake/p-h (four snakes found during 744.3 p-h of search). Snakes in riparian areas were found both in open and forested areas, mostly around gallery forests, natural lagoons, small pools, and palm marshes. We found no snake in wet areas (e.g., temporary pools and rivulets) located in interfluvial areas, far from gallery forests. We captured snakes in pitfall traps only during the wet season and always in riparian areas (1.6 snakes per 1000 bucket-days).

In all 26 observations during TCS, the snakes were exposed, either on the ground (92%;  $N = 24$ ) or on vegetation (8%;  $N = 2$ ); no specimen

was found sheltered or inside burrows and ground cavities. Both snakes found on vegetation were juveniles moving over twigs or grasses, 20 and 30 cm above ground, respectively. Eleven snakes (42%) were coiled in a typical ambush posture, 13 (50%) were moving, and two (8%) were apparently resting.

At IES, the encounter rate of TCS at night was 0.029 snake/p-h (16 snakes found during 547.0 p-h of search), whereas during daytime it was 0.005 snake/p-h (one snake found during 191.8 p-h of search). Also at IES, the encounter rate during the wet season was 0.030 snake/p-h (15 snakes found during 497.3 p-h of search), whereas during the dry season the encounter rate was 0.008 snake/p-h (two snakes found during 239.6 p-h of search).

Of the snakes obtained incidentally or by local collectors for which field data were recorded ( $N = 39$ ), 33 (84%) were in or close to riparian areas and on the ground, either resting or moving. Five were in water bodies. Two adult specimens were found moving over vegetation, up to 150 cm above ground, in or close to gallery forests, and only one snake was found in a burrow.

During TCS and incidental encounters, individuals of *B. moojeni* were active at air temperatures ranging from 18.5–25.0°C at night ( $N = 11$ ) and at 25.0 and 29.5°C during daytime ( $N = 2$ ). The number of snakes obtained from local collectors was higher-than-expected in the wet season and lower-than-expected in the dry season ( $\chi^2 = 4.37$ ,  $df = 1$ ;  $P = 0.04$ ). No climatic variable alone seems to explain the variation in the monthly number of snakes collected (maximum temperature,  $r^2 = 0.04$ ,  $F = 1.41$ ,  $P = 0.26$ ; minimum temperature,  $r^2 = 0.25$ ,  $F = 0.26$ ,  $P = 0.62$ ; rainfall,  $r^2 = 0.27$ ,  $F = 3.74$ ,  $P = 0.08$ ).

Sexual size dimorphism was evident in *B. moojeni* ( $U = 349.00$ ;  $P < 0.01$ ), with mature females (median 1034 mm; range 760–1330 mm;  $N = 55$ ) larger than mature males (median 827 mm; range 590–1060 mm;  $N = 45$ ). Mature females were heavier than males of similar size, with a relatively longer head, and a relatively shorter tail than mature males ( $F_{1,83} = 6.47$ ,  $P = 0.01$ ;  $F_{1,96} = 36.57$ ,  $P < 0.01$ ;  $F_{1,84} = 53.82$ ,  $P < 0.01$ , respectively).

*Feeding Habits.*—Of 207 dissected specimens, 127 (61.3%) contained prey remains in the gut. The percentage of snakes with food was 65.7% when only the 143 specimens obtained directly from the field are considered, of which 94 contained prey remains. Twelve snakes contained remains of more than one prey item in the entire gut, and only three of these had more than one prey item in the stomach. Five (15.1%) out of 33 prey items for which direction of ingestion could be detected were swallowed tail first.

TABLE 1. Contribution of prey groups to the diet of juveniles, mature males, and mature females of *Bothrops moojeni*.

Age group	With prey	No. of prey	Centipedes	Frogs	Lizards	Snakes	Mammals and birds
Juveniles N = 81	55 (68%)	60	0	30 (50.0%)	16 (26.7%)	2 (3.3%)	12 (20.0%)
Mature males N = 53	30 (57%)	35	1 (2.9%)	10 (28.6%)	6 (17.1%)	2 (5.7%)	16 (45.7%)
Mature females N = 73	42 (57%)	49	0	6 (12.2%)	4 (8.2%)	5 (10.2%)	34 (69.4%)
Totals 207	127	144	1	46	26	9	62

We recovered 144 prey items in six major taxonomic groups (Table 1): mammals (41.0% of all prey found), frogs (31.9%), lizards (17.4%), snakes (6.2%), birds (2.8%), and centipedes (0.7%). We detected an ontogenetic shift in prey types: ectotherms (mostly amphibians) are the main prey of juveniles whereas endotherms (mostly rodents) are the main prey of adults (Table 1; Fisher exact test,  $P < 0.01$ ). We also observed an intersexual difference in diet, with adult males consuming mostly ectotherms and adult females preying mostly on endotherms (Table 1; Fisher exact test,  $P = 0.02$ ). This difference was not evident (Fisher exact test,  $P = 0.12$ ) in a comparison between males and females of similar sizes (using a subsample ranging from the smallest mature female to the largest mature male).

We obtained prey mass for 28 partially or nondigested items. The ratio of prey mass: predator mass ranged from 0.008–1.079. The smallest prey was a juvenile lizard (*Tropidurus* sp.; 31 mm SVL, 1 g) eaten by an adult male *B. moojeni* (IB 61682, 700 + 132 mm, 120 g), and the largest an adult lizard *Ophiodes striatus* (Anguillidae; 136 mm SVL, 41 g) consumed by a juvenile female (IB 53139, 484 + 82 mm, 38 g). Prey mass was correlated with predator mass (adjusted  $r^2 =$

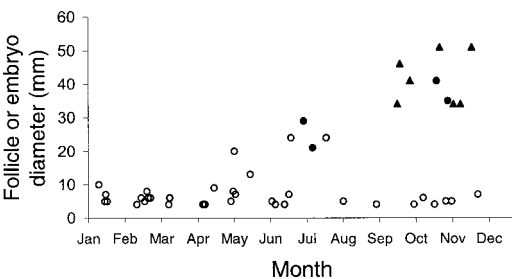
0.43,  $F = 21.66$ ,  $P < 0.01$ ,  $N = 28$ ). There was an ontogenetic shift in prey relative mass; the slope of the regression (0.66) of prey mass on predator mass was significantly different from one;  $t = 2.25$ ,  $P < 0.02$ ,  $N = 28$ , with juveniles tending to consume relatively heavier prey than adults.

We recorded tail-tip color for 126 snakes (31 juveniles, 95 adults). White tail tips occurred in most juveniles (70.9%) but were rare in adults (2.2%). Specimens having white or partially white tail tips ranged in size from the smallest individual (265 + 47 mm; CHUNB 03594, juvenile female) to an adult female (810 + 130 mm; IB 61686). Snakes with a tail tip color not different from the color of the remainder of the tail ranged from 305 + 46 mm (CHUNB 19296, juvenile female) to the largest individual (1330 + 180 mm; IB 60443, adult female).

*Reproduction.*—The reproductive cycle of *B. moojeni* was lengthy and seasonal (Fig. 1). The vitellogenic period starts around May, in the beginning of the dry season, with ovulation occurring around July. Births were concentrated around December and January, in the middle of the rainy season, as full-term embryos were present only in females collected in December. Some females collected during the breeding season had nonvitellogenic follicles, indicating that only part of the mature female population reproduces each year, although our sample was small. Litter size ranged from three to 32 young (mean  $15.6 \pm 7.9$ ,  $N = 21$ ) and was dependent on female body size (adjusted  $r^2 = 0.45$ ;  $F = 17.34$ ;  $N = 21$ ;  $P < 0.01$ ).

DISCUSSION

Habitat of *B. moojeni*, a relatively common species throughout the Cerrado, was poorly known until recently. Leloup (1984) was the first to suggest that the typical habitat of *B. moojeni* is riparian areas in the Cerrado. More recently, Borges and Araújo (1998) suggested that *B. moojeni* occurs mainly in and around gallery forests. Our results and those in Martins et al. (2002) strengthen these conclusions. The lack of



observations of *B. moojeni* around water bodies located in interfluvial areas corroborates the association of this pitviper to gallery forests. However, our study also indicates that *B. moojeni* can occasionally be found in dry interfluvial savannas. Although occupying the Cerrado domain, widely dominated by interfluvial savannas (Eiten, 1972), *B. moojeni* occurs mainly in forested habitats, as those occupied by other members of the *atrox* species group (e.g., *Bothrops atrox* and *Bothrops leucurus*; Martins et al., 2002).

*Bothrops moojeni* is active at relatively high air temperatures compared to sympatric congeners (e.g., *Bothrops alternatus* and *Bothrops itapetiningae* found active at 15 and 12°C, respectively, at IES, *B. alternatus* and *Bothrops neuwiedi pauloensis*, both found active at 13°C at ENP, and *Bothrops neuwiedi goyazensis* active at 14°C in the Brasília region; pers. obs.). Other species of the *atrox* group are also active at high air temperatures (e.g., *B. atrox*; Oliveira and Martins, 2001).

Our microhabitat data indicate that *B. moojeni* are occasionally found in the water of streams and ponds located in riparian areas (Leloup, 1984). Our observations also confirm that both juveniles and adults may occasionally be found moving on the vegetation (over twigs, grasses, and eventually higher branches; see Leloup, 1984; Martins et al., 2001). Similar observations were described for *B. atrox* in Amazonia (e.g., Martins and Oliveira, 1999).

In contrast, the use of ground cavities as retreats (see Leloup, 1984) was reported only once in our study, although the use of this microhabitat is commonly observed in other Cerrado species of *Bothrops* from interfluvial habitats (Borges and Araújo, 1998; Valdujo et al., 2002). The use of ground cavities may be an efficient way to reduce water loss and avoid exposure to predators and temperature extremes in open habitats (Vanzolini, 1948; Valdujo et al., 2002). However, gallery forests are wetter, more shaded, and show less variation in temperature than interfluvial areas, which may make the use of ground cavities less important for *B. moojeni*. Furthermore, ground cavities in riparian habitats are flooded during most of the year, making them unavailable to the snakes. The Amazonian *B. atrox* was observed using ground cavities on very few occasions (Martins and Oliveira, 1999).

Although limited, our mark-recapture data indicate that *B. moojeni* use small areas for long periods. The small number of *B. moojeni* captured in pitfall traps is probably caused by the low mobility of the species. Our results indicate that *B. moojeni* is mainly nocturnal and is active mostly during the warmest and wettest months (October to April), as are other species of *Bothrops* (Oliveira and Martins, 2002; Sazima, 1992; Valdujo et al., 2002).

Previous studies indicate that *B. moojeni* has a generalist diet (Leloup, 1984; Andrade et al., 1996). However, the relative contribution of prey categories in the diet of *B. moojeni* has not been determined, except for preliminary data provided by Martins et al. (2002). An ontogenetic dietary shift in *B. moojeni* (Martins et al., 2002; this study) was inferred previously by Andrade et al. (1996) from anecdotal information and generalizations based on the diet of related species. The diet of *B. moojeni* (Martins et al., 2002; this study) is similar to that observed for *B. atrox* (Martins and Gordo, 1993; Martins and Oliveira, 1999). These similarities in diet may reflect common ancestry, as well as similar prey availability in humid forests occupied by *B. atrox* and *B. moojeni*.

Sexual size dimorphism in *B. moojeni* (Vanzolini, 1991; this study) may be related to reproduction, since larger body size in females may lead to increased fecundity (Seigel and Fitch, 1984). Leloup (1975) observed births in *B. moojeni* from October to January, with over 60% of them in December, in agreement with our data. Leloup (1975) suggested that the gestation period in *B. moojeni* lasts about 200 days (see also Almeida-Santos and Salomão, 2002), which does not correspond strictly to the interval between copulation and birth (March through May and December through February, respectively; Almeida-Santos and Salomão, 2002; this study). Thus, fertilization is apparently delayed until June and July, when ovulation occurs (Almeida-Santos and Salomão, 2002; this study). Indeed, morphological changes that occur after copulation (uterine muscular twisting, see Almeida-Santos and Salomão, 2002), in the distal portion of the oviduct of female *B. moojeni*, indicate the occurrence of long-term sperm storage. A seasonal reproductive cycle is widespread in *Bothrops* (Almeida-Santos and Salomão, 2002), occurring even in species from less seasonal habitats, such as the Atlantic forest (*Bothrops jararaca*, Sazima, 1992; *Bothrops jararacussu*, O. A. V. Marques, pers. comm.) and the Amazon (*B. atrox*, M. E. Oliveira, pers. comm.). Recruitment is synchronic with a peak in amphibian abundance (Sazima, 1992; Andrade et al., 1996), which would ensure high food availability for neonate snakes. However, both snakes and their potential prey could respond to other environmental factors (rainfall, humidity, temperature, and any combination of these), with no causal relationship between prey abundance and snake recruitment (see Oliveira and Martins, 2002; Valdujo et al., 2002).

Our results indicate that *B. moojeni* in the Cerrado (a seasonal and relatively dry biome) are ecologically similar to other forest-dwelling

members of the *atrox* group, ranging from the Atlantic forest to Amazonia. These similarities may be caused by phylogeny or similarities between the habitats occupied by *B. moojeni* in the Cerrado and the forests occupied by other species of the *atrox* group, or both. The presence of gallery forests in the Cerrado may have provided suitable habitat for the dispersal (probably from northern forests) and maintenance of this forest-dwelling lineage in Cerrado areas of central and southeastern Brazil.

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## Behavioral and Ecological Adaptations to Water Economy in Two Plethodontid Salamanders, *Ensatina eschscholtzii* and *Batrachoseps attenuatus*

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**ABSTRACT.**—Amphibians in general, and plethodontid salamanders in particular, are highly susceptible to cutaneous water loss. We compared behavior, habitat use, and physiology of *Batrachoseps attenuatus* (Eschscholtz) and *Ensatina eschscholtzii* (Gray) in Humboldt County, California, to identify adaptations of water economy. Both species lost more body water in a dry environment than in a wet one. In the field, sites with salamanders had higher relative humidity than sites without salamanders. In behavioral experiments, both species used water-conserving postures; they positioned themselves deep in leaf litter in dry environments while remaining on the surface of leaf litter in wet environments. The slender morphology of *B. attenuatus* may aid in habitat partitioning by plethodontid salamanders; in the field we found *B. attenuatus* closer to the surface of the leaf litter than *E. eschscholtzii*. The evolution of a slender body shape appears to be unconstrained by microclimates typically encountered by this species.

The highly permeable skin of amphibians poses a serious risk of dehydration and death (Tracy, 1976). Despite the fact that taxonomic and life-history diversity within Amphibia is immense (Duellman and Trueb, 1986), the adaptive response to cutaneous water loss has been studied in only a few species. Of those species that have been studied, a diverse array of adaptive responses has been found, including

morphology (Winokur and Hillyard, 1992), posture (Stille, 1958), habitat selection (Jaeger, 1971), physiological mechanisms (Hillyard et al., 1998), and activity patterns (Gillis, 1979).

Plethodontid salamanders are uniquely challenged by cutaneous water loss. They are lungless, respiring via the cutaneous membrane and buccopharynx (Whitford and Hutchinson, 1967). Additionally, many plethodontids are entirely terrestrial, having no aquatic life stage (Bishop, 1943; Petranka, 1998). Resistance of the cutaneous membrane to water flow has not been well studied, although it is not thought to play a significant role in water conservation in sala-

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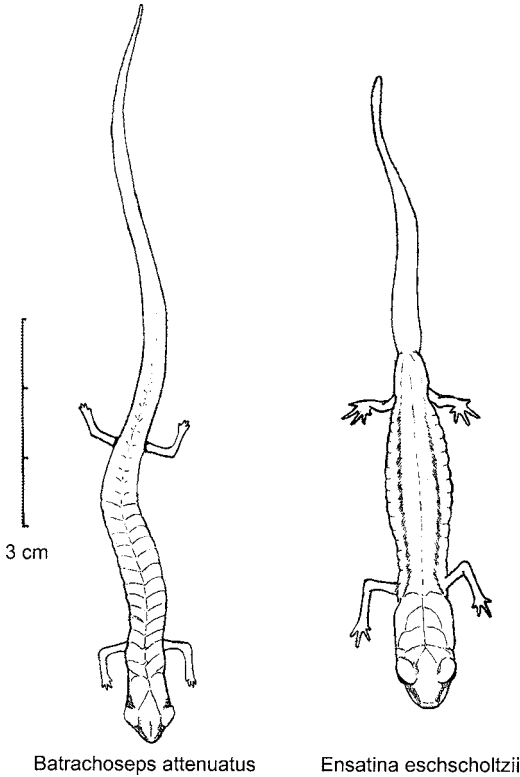


FIG. 1. *Batrachoseps attenuatus* and *Ensatina eschscholtzii* from Humboldt County, California, 1 September 1995.

manders. Thus, plethodontid salamanders are challenged with maintaining water balance in a desiccating environment with an integument that loses water at the same rate as a free water surface (Spotila and Berman, 1976). In this paper, we describe behavioral and ecological adaptations to water economy in two abundant species of plethodontid salamander. We also provide physiological data on cutaneous water loss and gain.

*Batrachoseps attenuatus* and *Ensatina eschscholtzii* are sympatric throughout much of their range and are habitat generalists (Bishop, 1943; Petranka, 1998). *Batrachoseps attenuatus* has a long slender body, reduced limbs, and a narrow head (Fig. 1), which enables it to maneuver through small spaces in leaf litter and soil. This species has often been observed in a coiled position, presumably to avoid dehydration (Cohen, 1952; Cunningham, 1960), but this behavior has not been quantified. *Ensatina eschscholtzii* has a more conservative morphology and larger mass (Fig. 1) and has not been reported to use water-conserving postures.

To better understand how each species responds to a desiccating environment, we (1) com-

pared micro- and macrohabitat characteristics in areas with and without salamanders; (2) quantified behavioral responses to varying environmental conditions; and (3) measured cutaneous evaporative water loss and gain in conditions that mimicked frequently encountered biophysical conditions in leaf litter of redwood forests.

#### MATERIALS AND METHODS

**Field Measures of Habitat Use.**—We measured biophysical conditions at two scales of habitat use within a redwood forest: ambient conditions within a stand of redwoods (stand), and under leaf litter (microsite). We searched for salamanders at 53 locations from 1 March 1995 to 3 September 1995 at Arcata Community Forest (ACF, 40°52'N, 124°03'W,  $N = 26$  plots) and at Korb, (40°55'N, 123°55'W,  $N = 27$  plots), approximately 8 and 18 km from the coast, respectively. ACF contained stands of 45–100-year-old redwoods (*Sequoia sempervirens*) and Korb contained 35–65-year-old stands of redwood mixed with Douglas-fir (*Pseudotsuga menziesii*). The predominant species in the understory of both sites were sword fern (*Polystichum munitum*), redwood sorrel (*Oxalis oregana*), and salal (*Gaultheria shallon*).

We chose the approximate location of sampling plots by viewing aerial photographs and choosing stands of trees that were predominantly redwood and were accessible by vehicle. We randomly determined the exact location for a plot within a stand; all plots were at least 40 m from roads and forest edge. Plots were 8 × 1 m and were placed along a randomly determined azimuth. We sampled one to three plots per day at randomly selected times within a 24-h period; we sampled each study area on alternate days to avoid temporal bias.

To sample a plot, leaf litter was carefully raked, and soil under the leaf litter was searched for salamanders to a depth of 3 cm. If a tree was inside the plot, bark was searched to a height of 1 m. We recorded counts of *E. eschscholtzii* and *B. attenuatus* and the depth ( $\pm 1$  mm) at which we found them below the exposed surface of the leaf litter. Each plot was classified as having only *B. attenuatus* present, only *E. eschscholtzii*, both species, or neither species.

We measured six biophysical variables (microsite and stand relative humidity, microsite and stand temperature, mean and maximum wind speed) at each plot. For the first salamander found at each plot, we removed the salamander and placed a sensor (Model HMP35C, Vaisala, Finland) at the microsite to measure relative humidity and temperature of the microsite; if we found no salamanders, sensors were placed at the center of the plot, six to nine cm below the

surface of the leaf litter to approximate a salamander microsite. We collected data 10 min after the instruments were deployed, so that instruments were equilibrated to their surroundings. We also measured relative humidity, temperature and wind speed at a height of 1 m above the forest floor in the middle of the plot to characterize the surrounding redwood stand. Stand relative humidity and temperature were measured with a sensor identical to the microsite sensor. Mean and maximum wind speed were measured with a cup anemometer (Model 03101-5, R. M. Young County, Travis City, MI). The cup anemometer had a threshold of 0.2 m/sec and could not measure wind less than the threshold. A datalogger (Model 21X, Campbell Scientific, Logan, UT) recorded data from the instruments at a scan interval of 1 sec; data were averaged at 2-min intervals and stored. These averages were again averaged over a 20-min period. Maximum wind speed was the greatest value recorded during the 20 min of data collection.

We used a Student's *t*-test to detect differences in the depth of substrate where individuals of each species were found. For this analysis, we considered each salamander the sampling unit regardless of its proximity to another salamander because we found no difference in biophysical variables at plots with either or both species of salamander (see Results). We used a two-way (season and salamander presence), multivariate analysis of variance (MANOVA) to test for species specific differences in biophysical variables (stand temperature, microsite temperature, stand relative humidity, microsite relative humidity) at plots. Season was defined as wet (from March through June) and dry (from July through September). Species specific differences were characterized by plots with only *B. attenuatus* present, only *E. eschscholtzii* present, both species present, or no salamanders present. We used a posthoc Tukey's HSD test to identify which plots were significantly different from one another in salamander presence. All biophysical variable distributions were tested for normality with a Kolmogorov-Smirnov Z-test for normality. If distributions were not normal they were transformed prior to conducting the MANOVA. We conducted all statistical tests of habitat use at an experiment-wide  $\alpha$  level of 0.05.

*Laboratory Measures of Behavior.*—We collected salamanders from our two field sites between February 1995 and December 1995. Salamanders were housed in outdoor facilities in a redwood grove at Humboldt State University Game Pens, Arcata, California at ambient light and temperature conditions encountered in a typical coastal redwood stand. Salamanders were held in translucent containers (20 × 30 × 15 cm) with leaf litter that was moistened daily and replaced weekly.

For *E. eschscholtzii* we provided two to three meal worms per week; uneaten mealworms were discarded at the end of a week. For *B. attenuatus*, we assumed that the freshly provided leaf litter contained detritus and small arthropods necessary to their diet. Forty-eight hours prior to conducting behavioral experiments, we placed individual salamanders in covered containers (15 × 15 × 15 cm) lined with paper towels saturated with distilled water without leaf litter or food.

We exposed individuals of both species to two moisture regimes (wet and dry) to determine whether water conserving postures were used by either species and to determine changes in the vertical location of salamanders in leaf litter in response to the two different moisture regimes. Prior to and immediately after each experiment, we weighed salamanders. We individually placed seven salamanders of each species into one of 14 laboratory arenas (15 × 15 × 15 cm) containing redwood leaf litter 3 cm deep. Leaf litter was collected from plots in ACF. Seven arenas contained wet leaf litter (leaf litter that had been submerged in distilled water for one hour). The other seven were dry arenas (leaf litter in these containers was submerged in distilled water for one hour and then dried to 70% of the wet weight). Pilot studies with drier leaf litter were lethal to salamanders. We placed three *B. attenuatus* and four *E. eschscholtzii* in the seven wet arenas (one salamander per arena) and four *B. attenuatus* and three *E. eschscholtzii* in dry arenas for 4 h. One week later the experiment was repeated with the same salamanders in opposite moisture regimes. All experiments were conducted in a temperature-controlled room at 0900 h PST at 20°C with indoor lighting.

We recorded posture and location in the leaf litter 4 h following placement of salamanders in the arenas. We categorized posture as curled when a salamander reduced exposed surface area by covering one area of its body with another part of its body. We categorized posture as straight when a salamander did not cover itself, thereby exposing all of its surface area to the environment. We categorized the vertical orientation of the salamander in the substrate as above the leaf litter or under the leaf litter. We used repeated measures log-linear analysis (Stevens, 1992) to examine the effect of the two moisture regimes on the posture and location of *B. attenuatus* and *E. eschscholtzii*. We used a two-factor analysis of covariance (ANCOVA,  $\alpha = 0.05$ ) to compare water loss (initial body mass minus final body mass) in each species on wet and dry substrates; initial body mass was the covariate.

*Laboratory Measures of Physiology.*—We measured rates of water loss and water gain in a controlled laboratory setting for *E. eschscholtzii*

TABLE 1. Mean  $\pm$  SE of biophysical variables measured in Humboldt County, California, during the wet season (March to June 1995,  $N = 27$ ) and the dry season (July to September 1995,  $N = 26$ ). We tested for differences in biophysical variables using a multivariate analysis of variance; season was one of two treatment groups (the other was salamander presence, Table 2).

Biophysical variable	Wet	Dry	$F_{1,55}$	$P$
Temperature ( $^{\circ}\text{C}$ )				
Stand	11.6 $\pm$ 0.6	17.4 $\pm$ 0.5	69.2	<0.001
Microsite	11.9 $\pm$ 0.4	16.4 $\pm$ 0.3	87.2	<0.001
Relative humidity (%)				
Stand	85.3 $\pm$ 1.7	75.2 $\pm$ 2.5	11.8	0.001
Microsite	91.5 $\pm$ 1.1	88.5 $\pm$ 1.6	24.4	0.04

( $N = 14$ ) and *B. attenuatus* ( $N = 7$ ). Twelve hours prior to beginning evaporative water loss experiments, we placed captive salamanders in a petri dish with a paper towel saturated with distilled water and then placed the petri dish inside a temperature controlled ( $\pm 0.1^{\circ}\text{C}$ ) chamber at  $12^{\circ}\text{C}$ . We assumed all individuals to be fully hydrated at the start of the experiment. Experiments began at 0900 h PST when experimental animals were temporarily paralyzed with gallamine triethiodide (Sigma Chemical Co., St. Louis, MO; 0.25 mg/ml gallamine triethiodide in 0.09% bacteriostatic saline solution) to ensure that animals did not use water conserving postures. This drug induces muscle end-plate paralysis and has no effect on cutaneous evaporative water loss in anurans (Bentley and Shield, 1973). *Batrachoseps attenuatus* and *E. eschscholtzii* received 0.2 cc and 0.4 cc, respectively, of drug administered topically (the small size of *B. attenuatus* salamanders precluded the use of injections). As soon as immobilization occurred, we rinsed salamanders with distilled water and weighed them ( $\pm 0.001$  g). Salamanders were then dehydrated at  $12^{\circ}\text{C}$  for 5 h in a closed airflow system which consisted of a Plexiglas chamber ( $7.5 \times 2.5 \times 1.0$  cm, inside dimensions) inside of a larger, temperature-controlled chamber. Anesthetized individuals were positioned ventral side down in an elongated position (Fig. 1). A constant flow of air (13.5 ml/min, STP) was passed through a silica gel filter and was measured with a glass bead flowmeter (No. 11, Gilmont Instruments, Inc., Barrington, IL). The silica gel reduced water vapor before air entered the Plexiglas chamber. Influent relative humidity was  $39.4\% \pm 2.5$  (mean  $\pm 1$  SE). In pilot studies, removing more water from the influent air damaged the cutaneous membrane of ani-

mals. We continuously measured relative humidity and temperature of effluent air with a relative humidity sensor (Model HMI32, Vaisala, Finland) placed in the airflow immediately after the chamber. Complete mixing of the influent air with water vapor from the salamander occurred in the fourth hour of the experiment for all individuals. Steady state was recognized as the stabilization of relative humidity of the effluent air (Bentley and Yorio, 1979). At the conclusion of 5 h, we reweighed salamanders to gravimetrically assess water loss, placed them in a covered petri dish with a paper towel saturated with distilled water, and then placed them back in the temperature-controlled chamber. We again reweighed salamanders to measure the amount of water gained after 3 h.

We used ANCOVAs with body mass as a covariate to test for relative differences in water loss and water gain between species on ln transformed data. We conducted all statistical tests for the physiology experiments at an  $\alpha$  level of 0.05.

## RESULTS

*Habitat Use.*—Biophysical variables were normally distributed with the exception of microsite relative humidity, which was arcsine transformed (Zar, 1984) to meet the assumption of normality. Mean wind speed within stands was rarely more than 0.2 m/sec and therefore was impossible to measure accurately. Microsite and stand temperatures, and microsite and stand relative humidity were measurably warmer and drier in the dry season (Table 1). Although plots without salamanders were significantly drier than plots with salamanders, a Tukey's HSD test revealed no differences in temperature or humidity in plots with only *E. eschscholtzii*, only *B. attenuatus*, or plots with both species (Table 2). Of 39 plots that contained salamanders, 41% ( $N = 16$ ) contained both species. We found *B. attenuatus* at a mean depth of  $6.6 \pm 0.4$  cm ( $N = 83$ ) from the litter surface, which was closer to the surface than *E. eschscholtzii* ( $9.1 \pm 1.0$  cm,  $N = 40$ ;  $t = 2.56$ ,  $P = 0.012$ ,  $df = 118$ ).

*Behavior and Physiology.*—Both *B. attenuatus* and *E. eschscholtzii* curled more often in the dry than in the wet environment, and were straight more often in the wet than the dry environment, and both species were under leaf litter more often in the dry than in the wet environment (Table 3). Both species lost more water in the dry substrate than in the wet substrate ( $F_{1,23} = 4.50$ ,  $P = 0.04$ ), but we found no significant difference in water loss (standardized for body mass) between species ( $F_{1,23} = 0.08$ ,  $P = 0.78$ ;  $0.23 \pm 0.07$  g for *B. attenuatus* in the dry substrate and  $0.15 \pm 0.07$  g in the wet substrate; for *E. eschscholtzii* water loss was  $0.23 \pm 0.06$  g in the dry substrate and  $0.08 \pm 0.06$  g in the wet substrate). Even when con-

TABLE 2. Biophysical variables (mean  $\pm$  1 SE) for plots with only *Batrachoseps attenuatus* present ( $N = 12$ ), only *Ensatina eschscholtzii* present ( $N = 11$ ), both species present ( $N = 16$ ), and no salamanders present ( $N = 14$ ) in Humboldt County, California. A Tukey's HDS test revealed that plots without salamanders were drier at the stand and microsite scale than plots with salamanders.

	<i>B. attenuatus</i> only	<i>E. eschscholtzii</i> only	Both species	Neither species	$F_{3,55}$	$P$
Temperature						
Stand	14.9 $\pm$ 1.3	13.9 $\pm$ 1.0	13.9 $\pm$ 0.7	15.1 $\pm$ 1.5	0.4	0.76
Microsite	14.5 $\pm$ 1.0	13.7 $\pm$ 0.6	13.7 $\pm$ 0.6	14.6 $\pm$ 1.1	0.6	0.57
Relative humidity						
Stand	81.3 $\pm$ 3.5	85.7 $\pm$ 2.0	82.2 $\pm$ 1.9	73.3 $\pm$ 4.3	3.5	0.02
Microsite	90.3 $\pm$ 1.1	92.1 $\pm$ 3.3	92.3 $\pm$ 1.5	85.6 $\pm$ 2.6	4.2	0.009

ditions were standardized in the laboratory, we found no significant difference in mass-specific water loss ( $F_{1,18} = 0.61$ ,  $P = 0.45$ ) or water gain ( $F_{1,18} = 0.83$ ,  $P = 0.36$ ) between species. A power analysis of water loss data revealed low power ( $1-\beta = 0.11$ ). Absolute cutaneous water loss was  $0.071 \pm 0.008$  g/5 h for *B. attenuatus* with a mean mass of 0.71 g and  $0.090 \pm 0.007$  g/5 h for *E. eschscholtzii* with a mean mass of 1.98 g. Absolute water gain was  $0.15 \pm 0.29$  g/3 h for *B. attenuatus* and  $0.33 \pm 0.006$  g/3 h for *E. eschscholtzii*.

#### DISCUSSION

*Adaptations to Cutaneous Water Loss.*—The amount of moisture available to an amphibian has a potentially direct consequence to energy expenditure and locomotion. For example, in *Eleutherodactylus coqui*, a leptodactylid frog, elevated water loss rates resulted in increased resting oxygen consumption and decreased locomotor performance (Pough et al., 1983), a condition that may decrease survival in the wild. In the laboratory, we found that salaman-

ders exposed to dry conditions lost more body water than when in wet conditions suggesting that dry substrates decrease survival in salamanders. One adaptation to cutaneous water loss is to avoid dry environments, and indeed, we found that on both a macro- and a microhabitat scale, salamanders avoided dry conditions. In the field, we found that plots with no salamanders were on average drier, both within the leaf litter and above the leaf litter, than plots with salamanders suggesting that salamanders require some minimum level of relative humidity to occupy a habitat. In the laboratory, both species behaviorally modified the moisture potential of their environment by burrowing under leaf litter when exposed to a dry environment. Other amphibian species restrict surface activity to times when there is precipitation, standing water, or increased relative humidity (Loveridge, 1977; Keen, 1984; Cree, 1989). Our behavioral experiments illustrate the ability of plethodontid salamanders to use microsites that minimize water loss.

TABLE 3. Contingency table of repeated measures experiment in which number of *Ensatina eschscholtzii* ( $N = 7$ ) and *Batrachoseps attenuatus* ( $N = 7$ ) were categorized as curled or straight and under or on top of leaf litter when placed in wet and dry moisture regimes, Humboldt County, California. We used log linear analysis to test for differences in behavioral categorizations (posture or position) between species and between moisture regimes. We found no difference in behavior between species, however both species behaved differently in wet versus dry moisture regimes. Each  $\chi^2$ -value represents a significant test of partial correlation for the category Wet or Dry substrate for posture or position.

	<i>B. attenuatus</i>		<i>E. eschscholtzii</i>		$\chi^2$	$P$
	Posture					
	Curled	Straight	Curled	Straight		
Wet	2	5	0	7	9.93	0.005
Dry	7	0	6	1	12.20	<0.001
	Position					
	Underneath	On top	Underneath	On top		
Wet	3	4	3	4		
Dry	6	1	6	1	12.20	<0.001

It has been reported anecdotally that *B. attenuatus* uses water-conserving postures, but we found no reference for such behavior in the literature for *E. eschscholtzii*. We found that both species used surface area reducing postures in a dry environment. This behavior is not uncommon in amphibians; anurans increased their exposed ventral surface area to moist substrates and decreased the amount of exposed surface area when in contact with dry substrates (Stille, 1958; Pough et al., 1983; Brekke et al., 1991). In *E. coqui*, water-conserving postures prevented potentially lethal rates of dehydration, which allowed frogs to forage in highly variable conditions of the forest canopy (Pough et al., 1983). Furthermore, demognathine salamanders are reported to decrease clutch desiccation by coiling tightly about their eggs (Forester, 1984).

*Morphology and Habitat Use.*—In some species of plethodontid salamander, the moisture gradient across the environment constrains habitat use (Jaeger, 1971). We hypothesize that the specialized morphology of *B. attenuatus* did not constrain habitat use in redwood forest as expected if this species lost relatively more water than *E. eschscholtzii*. Although water loss experiments were inconclusive because of low power in the analysis, habitat use data suggest that both salamander species use similarly moist redwood stands. On a microsite level of habitat use, however, we found that *B. attenuatus* used leaf litter on average 3 cm closer to the desiccating surface than *E. eschscholtzii*. It appears that instead of constraining habitat use, the long slender body shape of *B. attenuatus* enables this species to more easily maneuver and forage at shallower depths of leaf litter than *E. eschscholtzii*. This habitat partitioning may allow each species to use different prey and spatial resources. Indeed, in a previous study these salamanders chose prey that differed in mean and maximum size (Lynch, 1985). Additionally, the morphology of *B. attenuatus* may allow it to use smaller refugia than *E. eschscholtzii*. Although ultimate causes of morphological differentiation and habitat use are unknown, physiological constraints of water economy do not appear to play a primary role.

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## Ambient Temperature, Activity, and Microhabitat Use by Ornate Box Turtles (*Terrapene ornata ornata*)

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**ABSTRACT.**—We examined the relationships between ambient temperature, activity level, and vegetation structure of microhabitats used by Ornate Box Turtles; vegetation structure of these habitats should influence microclimatic conditions and, thus, the thermoregulatory value of microhabitats. Thirty-four Ornate Box Turtles (*Terrapene ornata ornata*) were radio-tracked during 1997 and 1998 in the Nebraska Sandhills. Activity level was negatively related to ambient temperature. Active turtles used areas with more bare ground in both years, and more forb cover in one year, whereas inactive turtles used areas with more shrub and litter cover. Providing habitat components that function as thermoregulatory cover may be an important consideration for land management plans in areas where this species is found.

The Ornate Box Turtle (*Terrapene ornata ornata*) is a small, terrestrial emydid turtle distributed throughout the southern and central Great Plains. In Nebraska, the species is found predominantly in the Sandhills ecosystem (Lynch, 1985), a complex of vegetation-stabilized sand dunes encompassing approximately 5 million ha in the center of the state (Bleed and Flowerday, 1989). Concern over population levels of all box turtle species in North America led to the listing of these species in 1994 under Appendix II of the Convention on International Trade of En-

dangered Species of Wild Fauna and Flora (CITES; U.S. Fish and Wildlife Service, 1995).

Because of the physiological constraints of ectothermy, ambient temperature has an important effect on activity levels in box turtles (Legler, 1960; Gatten, 1974; Adams et al., 1989). Activity level was found to be negatively related to ambient temperature in Desert Box Turtles (*Terrapene ornata luteola*), resulting in bimodal activity patterns during the summer, in which turtles were active in the morning and afternoon and inactive during midday (Nieuwolt, 1996). Ornate Box Turtles in Kansas exhibited a similar bimodal activity pattern, and the midday inactive period was lengthened during the hottest portions of the summer (Legler, 1960). This pattern was also found in Ornate Box Turtles in western Nebraska, where turtles were most often active from 0600–1000 h and 1600–1800 h

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(Converse et al., 2002). Based on this evidence, it is likely that optimal thermal conditions for ornate box turtles do not exist during the hottest part of summer days, at least over much of the species' range, and a negative relationship exists between activity level and ambient temperature during summer months.

Outside of optimal thermal conditions, ectothermic species must resort to behavioral thermoregulation to avoid body temperatures that are physiologically intolerable. Therefore, thermoregulatory requirements should influence not just activity levels of box turtles, but microhabitat selection as well, because behavioral thermoregulation consists of the selection of optimal microclimates (Adams and DeCarvalho, 1984). Microclimatic factors, such as temperature and moisture conditions, should be the driving factors in selection of thermoregulatory sites, and have been demonstrated to be important environmental components of shallow excavated pits, known as forms (Stickel, 1950), used by inactive Three-Toed Box Turtles (*Terrapene carolina triunguis*; Reagan, 1974). However, appropriate microclimatic conditions may ultimately be determined by vegetation structure; thus the structural characteristics of microhabitats may be critical in providing thermoregulatory cover (Converse, 1999). Associations have been found between form locations of Three-Toed Box Turtles and structural factors including percent total ground cover, percent total canopy cover, and percent litter cover (Reagan, 1974). Further anecdotal evidence lends support for a link between thermal conditions and microhabitat use in Ornate Box Turtles. Ornate Box Turtles in Kansas sought shelter in shaded areas during the midday inactive period when temperatures were highest (Legler, 1960). However, we know of no quantitative studies on the structural components of microhabitats used by Ornate Box Turtles and how these relate to thermoregulatory behaviors.

If ambient temperatures have a negative influence on activity levels, and turtles seek thermoregulatory cover during periods of high temperature, there should be a link between activity level and structural characteristics of the microhabitat. Limited evidence is available for such a relationship. For example, inactive Desert Box Turtles in New Mexico used kangaroo rat (*Dipodomys* spp.) burrows and clumps of vegetation (Nieuwolt, 1996). Furthermore, the characteristics of the microhabitats used by inactive turtles should be indicators of habitat components that provide thermoregulatory cover. In this study, we first tested the hypothesis that ambient temperature has a negative effect on activity level in our study area during the summer months. We then tested the hypothesis

that activity level is related to microhabitat use. We examined the structural components of microhabitat that were used by inactive turtles in an attempt to understand components of microhabitats that are important as thermoregulatory cover.

#### MATERIALS AND METHODS

*Study Area.*—Research was conducted at Crescent Lake National Wildlife Refuge, Garden County, Nebraska. The refuge is located in the western portion of the Nebraska Sandhills and comprises approximately 18,000 ha. Upland sites dominate in the eastern portions of the refuge. A mix of upland and meadow sites, as well as a high density of wetlands with associated emergent vegetation, occurs in the central part. A mix of upland and meadow sites is found in the west. Dominant plants included cattails (*Typha* spp.) and bulrushes (*Scirpus* spp.) in the emergent zone; sedges (*Carex* spp.), switchgrass (*Panicum virgatum*), and Kentucky bluegrass (*Poa pratensis*) in the meadows; and sand bluestem (*Andropogon hallii*), needle-and-thread (*Stipa comata*), hairy grama (*Bouteloua hirsuta*), prairie sandreed (*Calamovilfa longifolia*), sand lovegrass (*Eragrostis trichodes*), and little bluestem (*Schizachyrium scoparium*) in the uplands. Moreover, uplands on parts of the refuge had high densities of yucca (*Yucca glauca*), an evergreen shrub. Weaver (1965), Gunderson (1973), and Iverson (1991) provided further descriptions of the study area. During this study (1997 and 1998), mean annual temperature on the refuge was 10.1°C, and mean annual maximum temperature was 39.2°C (M. G. French, U.S. Fish and Wildlife Service, pers. comm.).

*Capture and Radio-Telemetry.*—In 1997, turtles were initially caught through incidental encounters on the refuge, including on roads. However, we quickly became concerned that capturing turtles in this way would have the potential to introduce bias into the habitat measures. Therefore, additional turtles in 1997 and all turtles in 1998 were captured at study sites established randomly on the refuge. Twelve study sites, each having a 200-m radius, were established. Each site was centered on an intersection of section lines and selected from a random sample of 25 based on accessibility, as well as to facilitate location of sites in different representative macrohabitat types. Sites were located in three broad macrohabitat types: upland ( $N = 5$ ), meadow ( $N = 2$ ), and transitional (between uplands and meadows,  $N = 5$ ). Turtles were captured during intensive searches of the study sites by researchers and researchers working with Labrador retrievers (*Canis familiaris*; Schwartz and Schwartz, 1974). In 1998, in addition to searches,

TABLE 1. Means, standard errors (SE), confidence intervals, and *P*-values for tests of differences between microhabitat used by active and inactive Ornate Box Turtles on Crescent Lake National Wildlife Refuge during summers 1997 and 1998.

Habitat component	<i>P</i>	Inactive		Active	
		Mean (SE)	Confidence limits	Mean (SE)	Confidence limits
% Bare ground	0.054	31.58 (1.97)	(27.70, 35.45)	38.51 (3.39)	(31.84, 45.18)
% Grass	0.598	43.05 (2.35)	(38.43, 47.67)	44.77 (3.44)	(38.00, 51.54)
% Forb (1997)	<0.001 <sup>a</sup>	9.09 (1.26)	(6.62, 11.56)	26.26 (2.60)	(21.15, 31.37)
% Forb (1998)	0.661 <sup>a</sup>	14.97 (1.77)	(11.49, 18.45)	13.32 (3.35)	(6.73, 19.90)
% Shrub	<0.001	18.68 (2.04)	(14.67, 22.70)	2.39 (3.36)	(0, 9.00)
% Litter	0.066	43.06 (1.62)	(39.87, 46.25)	37.22 (2.92)	(31.47, 42.97)
Vegetation height (cm)	0.309	36.83 (1.40)	(34.07, 39.59)	33.86 (2.64)	(28.66, 39.06)

<sup>a</sup> *P*-values presented for forb cover are not based on the model but are from tests of the differences of the least-squares means in each year.

turtles were captured at drift fence arrays placed in the study sites. One drift fence array was located in each study site, centered as close as possible to the intersection of the section lines that defined the center of the study area. The drift fence array design, modified from Campbell and Christman (1982), consisted of three 9.2-m lengths of 30.5-cm (1-ft) high, 0.6-cm (0.25-in) mesh hardware cloth, with two rectangular funnel traps at the end of each. The funnel traps were constructed of 0.6-cm mesh hardware cloth, and measured 30.5 cm long by 22.9 cm wide by 22.9 cm high. During both years, approximately equal effort was made to capture turtles in each of the 12 study sites.

Standard methods (e.g., Legler, 1960) were used for measuring carapace length, plastron length, interabdominal seam length, and body mass for each turtle captured. Age class (juvenile or adult) was determined, and an estimate of age was made for juveniles and young adults by counting growth rings. Turtles were assumed to be juveniles if they were smaller than approximately 110 mm carapace length, had no obvious secondary sex characteristics, or had fewer than 13 growth rings (Converse, 1999). Sex was determined as described in Legler (1960). All turtles were individually marked by notching the carapace with a metal file or small hacksaw blade using a system adapted from Cagle (1939).

A sample of the captured turtles was fitted with 13.5-g transmitters (Advanced Telemetry Systems, Isanti, Minnesota) equipped with a thermistor that caused the transmitters to cease transmission after seven consecutive days of average daily air temperatures below 5°C, thus saving battery life. Transmitters resumed transmission in the spring after seven consecutive days of average daily air temperatures above 5°C, allowing turtles to be captured and transmitters removed before batteries failed. A silicone sealant was used to affix the transmitters to the back of the carapace. Turtles were

then released at the same location where they were captured.

Fieldwork was conducted from 29 May to 16 August 1997 and from 7 May to 19 August 1998. Turtles were generally located during the field season at least once per week with a hand-held receiver and a three-pronged yagi antenna (Wildlife Materials Inc., Carbondale, Illinois). Visual contact was made with turtles when possible, but not when turtles were located in underground burrows or in very dense meadow grasses.

When turtles were located, time and activity were noted, and the location was marked for future data collection. Activity was classified into the following categories based on visual observation: surface active (moving); surface inactive (inactive above the soil surface); inactive in form (inactive in a shallow excavated pit partially below the soil surface, as defined by Stickel, 1950); inactive in burrow (inactive in an abandoned burrow, created by a badger [*Taxidea taxus*], kangaroo rat [*Dipodomys ordii*], or other species); and unknown. Activity was then more generally classified for analyses as either active or inactive; locations where activity was unknown were not used in the analyses. These definitions have previously been used by Nieuwolt (1996) to characterize box turtle activity level, that is, inactive defined as below ground or stationary and sheltered by vegetation above ground.

*Microhabitat Analysis.*—Microhabitat analysis was conducted at all locations of radio-tagged turtles. When turtles were in the same location on consecutive sightings, data were gathered only for the first sighting, because of a lack of independence between consecutive sightings at the same location. Habitat data were gathered in a 50 × 20 cm Daubenmire frame (Daubenmire, 1959) centered over the capture site. Frames were oriented over locations so that the long axis faced north. Within the frames, cover analysis, in-

cluding percent bare ground, grass, forb, shrub, and litter, was conducted. Vegetation height also was recorded at five points along a transect running along the center of the long axis of the Daubenmire frame, at 5, 15, 25, 35, and 45 cm from the left side of the frame; these five readings were averaged. Hourly air temperature readings were obtained from a refuge-based monitoring station and were matched by time with each turtle sighting.

*Statistical Analyses.*—We used the NLMIXED procedure in SAS software (Vers. 8.02, SAS Institute, Inc., Cary, North Carolina) to model the effect of temperature on activity level. We constructed a global model with an intercept, year, temperature, and a year by temperature interaction. We first examined the 95% confidence interval on the interaction effect and then on the year effect; we eliminated these terms from the model because the confidence intervals included 0.

We used the MIXED procedure in SAS software (Vers. 8.02, SAS Institute, Inc., Cary, North Carolina) to model the effect of activity level on habitat use. We specified a model with activity level, year, and the activity level by year interaction term; individual turtle nested within year was modeled as a random effect.

#### RESULTS

A total of 144 individual turtles were captured during the study. Of these, 34 were fitted with transmitters and radio-tracked. Nineteen turtles (12 females, six males, and one juvenile) were fitted with transmitters in 1997. Of these, 13 were initially captured off of the randomly selected study sites, whereas six were captured on the study sites. Because of our concern for potential bias associated with non-random sampling of the turtles, we chose to continue tracking in 1998 only those turtles that were captured on study sites. Transmitters were replaced on four of these turtles (one female and three males) in the spring of 1998 and they were tracked through the 1998 season. Loss of transmitters between the end of the 1997 field season and the spring of 1998 precluded continued tracking of two additional turtles. In 1998, 15 additional turtles (12 females, two males, and one juvenile) were fitted with transmitters; these turtles were captured exclusively on study sites.

Turtles were observed at four of five of the randomly selected study sites classified as uplands and at three of five study sites classified as transitional. Turtles were never observed at the two study sites classified as meadows. We did not quantify macrohabitat type used by turtles, although observations indicated that radio-tracked turtles spent the majority of time in upland sites. However, radio-tracked turtles

were observed to use wet meadows on occasion and were also observed using shallow pools and lake margins. Use of water bodies was most common during May by nesting females and in hot periods of July by both sexes.

Microhabitat use was recorded at a total of 489 unique radio-locations between 29 May and 16 August 1997 and between 7 May and 19 August 1998. We failed to make visual contact with animals at 33 of these locations, and activity level was classified as unknown at an additional 20 locations. Therefore, activity of turtles was determined at 436 locations, and these locations were used in the analyses. The majority of observations at these locations (344 of 436, 79%) were of inactive turtles.

As we hypothesized, temperature had a negative influence on activity level. The estimate of the effect of temperature on activity level was  $-0.17$  (SE = 0.03; 95% CI =  $-0.24, -0.01$ ;  $P < 0.01$ ). Exponentiation of the effect size produces an odds ratio for activity. For example, the odds of a turtle being active at 25°C divided by the odds of a turtle being active at 24°C is equal to 0.84 ( $e^{-0.17} = 0.84$ ).

As hypothesized, activity level in turn had an effect on habitat used by turtles. The percent forb cover (in 1 yr) and percent shrub cover used varied significantly by activity level ( $P < 0.05$ ). There was also evidence that percent bare ground and percent litter used varied by activity level ( $P < 0.10$ ). Active turtles used areas characterized by greater proportions of bare ground, and lesser proportions of shrub and litter cover (Table 1). Use of forbs was greater by active turtles than inactive turtles in 1997; in 1998, no difference was found for forb use (Table 1). A significant year by activity interaction term ( $P < 0.05$ ) was found for forb cover, so least-squares means are reported by year for this variable (Table 1). A significant interaction term was also found for shrub cover, although the directionality of the relationship did not change from 1997 to 1998 between active and inactive turtle use of shrubs. As a result, we present the means across years for this variable (Table 1).

#### DISCUSSION

Turtles in the present study displayed bimodal activity patterns during the summer months, with higher activity levels early and late in the day. We found a negative relationship between activity level and ambient temperature, which suggests that the bimodal activity patterns observed are a result of temperature limitations during the hottest periods of the day. Similar bimodal activity patterns have been observed in Ornate Box Turtles in Kansas (Legler, 1960), in Desert Box Turtles in New Mexico (Nieuwolt, 1996), and in Florida Box Turtles (*Terrapene*

*carolina bauri*; Dodd et al., 1994). This is the first study linking these bimodal activity patterns to a negative relationship between activity level and temperature for Ornate Box Turtles.

Furthermore, in our study, active and inactive Ornate Box Turtles used distinct microhabitats. In particular, activity level had a strong relationship to the amount of shrub cover used. The Sandhills uplands, where turtles spent most of their time, are characterized by sparsely vegetated sandy soil, interspersed with an evergreen shrub, yucca (*Yucca glauca*), the major shrub species at Crescent Lake. Yucca are generally >0.75 m in diameter, with a simple rooting system consisting of a large taproot. Therefore, most of the area under the shrub is root-free and provides a substrate for excavation of forms. Although we did not record data on specific plant species, nearly all observations of shrubs used by Ornate Box Turtles were of yucca. Ornate Box Turtles in New Mexico were also observed using various shrubs for cover, including creosote bush (*Larrea divericata*), tarbush (*Flourensia cernua*), yucca (*Yucca elata*), and mesquite (*Prosopis juliflora*; Norris and Zweifel, 1950).

Activity level in ectothermic species such as box turtles is an important determinant of individual life histories, population dynamics, and species distribution because of the effect of activity on survival, growth, and reproduction (Curtin, 1995, 1998). Quickly changing thermoregulatory needs, that is, over the course of a summer day, require that thermal cover be available near areas where activities, such as feeding and reproductive behaviors, occur. Although box turtles require vegetation cover to support thermoregulation, adequate amounts of open sand are also required to support the digging of nests and hibernacula and, possibly, feeding (Converse, 1999). Therefore, the availability and arrangement of thermal cover throughout the habitat is almost certainly a determinant of population success. Yucca, an apparently important source of thermoregulatory cover in the Nebraska Sandhills, is believed to increase under heavy grazing (Collins, 1971), but there is little understanding of the impact of fire on yucca. Both grazing and fire have played a significant historical role in shaping Sandhills vegetation communities (Bragg and Steuter, 1996). Maintenance of the ecological processes under which these vegetation communities developed may be important to support box turtle populations. More study of these processes is needed to determine the potential impacts of current management on these vegetation communities and, in turn, on box turtles.

Water is an additional component of habitat that may be important for behavioral thermoregulation by Ornate Box Turtles and should be

examined further in this context. Based on our anecdotal observations, it is likely that water bodies are important for thermoregulation in very hot periods of the summer and, along with shrubby vegetation, should be considered in management plans for Ornate Box Turtles. Although Ornate Box Turtles have generally been perceived as highly independent of water (Hudson, 1985), the literature contains multiple observations of Ornate Box Turtles using water (Clarke, 1950; Norris and Zweifel, 1950; Blair, 1976; Dodd, 2001).

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