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THE EFFECT OF TEMPERATURE AND INTERSTIMULUS INTERVAL ON HABITUATION IN EARTHWORMS (*LUMBRICUS TERRESTRIS*)

AARON RIESMEYER and JOHN H. HULL, Bethany College, Bethany, WV 26032

ABSTRACT

Earthworms initially were housed at 21° C, then exposed to three days of habituation training to a vibratory stimulus. Between days of habituation training, randomly-selected groups of worms were housed at 7° C or 21° C. During habituation training, a random half of the worms in each housing temperature group had an interstimulus interval (ISI) of 10 sec, the other half an ISI of 20 sec. Prior to daily habituation sessions, all earthworms were warmed to 21° C. A repeated-measures ANOVA performed on number of stimulus presentations needed to produce habituation showed a significant effect of housing temperature; worms maintained at 21° C showed significant decreases in number of stimulus presentations needed to produce habituation across the three days, while those housed at 7° C did not. ISI did not produce any significant main or interactive effects. These results extend to an ectothermic species the prior finding in an endothermic species that cooling an organism immediately after training sessions may interfere with formation and/or retrieval of long-term memory, but do not support in earthworms faster habituation with a shorter ISI.

INTRODUCTION

Humans and nonhumans exhibit stereotypical startle responses to various stimuli, including loud noise and tactile stimulation (Rankin, Beck, & Chiba, 1990; Staddon, 1993). If the stimulus is not actually harmful to the organism, the intensity and predictability of the response will diminish as the stimulus is repeated. This gradual elimination of the response with repeated exposures to the stimulus, known as habituation, is among the most elementary forms of learning.

In part because of ethical concerns about the use of vertebrates in psychological research, many studies conducted in recent years in the area of learning and memory have involved invertebrates. Extensive information available about the anatomy, developmental history, and genetics of many species of invertebrates, including various worms and jellyfish, has made them ideal subjects for studies involving learning in general, and habituation in particular (Arbit, 1957; Johnson & Wuensch, 1994; Rankin, Beck, & Chiba, 1990). For example, Rankin and others (Broster & Rankin, 1994; Rankin, Beck, & Chiba, 1990; Rankin & Broster, 1992) have extensively studied habituation in the small nematode *Caenorhabditis elegans*, showing that it will habituate more quickly to a vibratory stimulus when the interstimulus interval (ISI) is shorter, recover more quickly from the effects of habituation when the ISI is shorter, and habituate more quickly when the ISI is constant as opposed to variable.

Another line of study has focused on temperature and its effects on learning and retention in vertebrates. Studies of rats have shown that inducing hypothermia shortly after passive avoidance training will result in poorer performance of that response in the future, unless a "reminder" cooling is administered immediately before the retention test (Hinderliter, Webster, & Riccio, 1975; Mactutus & Riccio, 1978). Although researchers using invertebrates as subjects frequently report temperature maintained during experimentation (e.g., 20° C in Broster & Rankin, 1994, and Rankin & Broster, 1992), or between experimental sessions (e.g., refrigerated in Arbit, 1957), the effect of temperature on rate of habituation or recovery from habituation has not been studied systematically.

In our study, we attempted to replicate and extend to earthworms many of the findings of Rankin and others using *C. elegans*, since pilot research in our laboratory had shown that worms habituate readily to a vibratory stimulus.

Further, we systematically varied housing temperatures maintained between daily sessions to see whether lower temperatures would result in poorer performance of a habituated response in an ectothermic species.

MATERIALS AND METHODS

Subjects and Apparatus

Subjects were 20 earthworms (*Lumbricus terrestris*) obtained from a local bait supplier. For seven days before daily sessions began, worms were housed individually at a room temperature of 21° C in Styrofoam cups containing properly moistened worm bedding. Between daily sessions, 10 worms were housed in a refrigerator maintained at 7° C, while the other 10 were maintained at a room temperature of 21° C.

All habituation sessions involved the same experimental apparatus delivering the same vibratory stimulus. An ordinary 6-V doorbell was mounted on a piece of hardboard 3 mm thick and 45 cm^2. A piece of transparent plastic tubing 11 mm internal diameter and 75 cm long, with 2 mm air holes drilled every cm, was attached to the hardboard and to the bell. Transparent tubing allowed continuous monitoring of worm behavior throughout experimental sessions, while air holes not only provided ventilation, but because of their spacing a means of gauging degree of worm contraction to the vibratory stimulus. Before each worm's habituation session, the tubing was rinsed in distilled water, then reattached to the hardboard and bell. The doorbell was rung, and a vibratory stimulus thereby administered, by use of a hand-held switch.

PROCEDURE

Half of the worms (n = 5) in each housing temperature condition received habituation trials with a 10-sec ISI, while the other half received habituation trials with a 20-sec ISI. For each habituation session, a worm was placed in the moistened tube for 1 min prior to the beginning of habituation trials. At the end of a particular worm's ISI, vibratory stimulation was administered for 1 sec, as a new ISI began. Habituation trials continued until each worm met a criterion of three successive trials without a response. Responding was defined as cessation of crawling, or a contraction, if the worm was crawling when the vibratory stimulus began, or a contraction if the worm was not crawling when the vibratory stimulus began. After reaching the nonresponse criterion, the worm was removed from the experimental apparatus, placed in its individual housing cup, and stored at either 7° C or 21° C. All worms in the 7° C storage cups were given a 1-hr warming period at 21° C immediately prior to both remaining daily sessions. Sessions continued for a total of three days. The experimenter was careful to remain as motionless as possible during sessions, and the experimental apparatus was in indirect, artificial light during all sessions.

RESULTS

A 2 (10-sec vs 20-sec ISI) x 2 (storage temperature of 7° C vs 21° C) x 3 (experimental days) repeated-measures analysis of variance was conducted on trials to habituation, and Newman-Keuls posttests, where appropriate, were used. Since no statistically significant main or interactive effects of ISI were found, ISI data are not included in Table 1. There was a significant effect of experimental days, $F(2, 32) = 20.32$, $p < .001$, and a significant experimental days by storage temperature interaction, $F(2, 32) = 8.20$, $p = .001$. Posttests revealed that worms housed at 21° C between experimental days showed a statistically significant decline in trials to habituation from day 1 to day 2, and from day 2 to day 3, while the worms housed at 7° C did not show any significant changes in trials to habituation across days. These results are summarized in Table 1.

Table 1

Mean trials to habituation and 95% confidence intervals (CI) across three experimental days for worms housed at 7° C or 21° C between experimental days

	Day 1	Day 2	Day 3
7° C	28.0 (CI = 25.1-30.9)	26.0 (CI = 23.0-29.0)	25.7 (CI = 23.1-28.3
21° C	31.3 (CI = 28.4-34.2)	27.5 (CI = 24.5-30.5)	21.8 (CI = 19.2-24.4)

DISCUSSION

The present study shows that the process of habituation in earthworms may be somewhat different from the process of habituation in *C. elegans*. For example, in the present study doubling the ISI had no significant effect on trials to produce habituation. However, Broster and Rankin (1994), working with *C. elegans*, used ISIs of up to 60 seconds, six times as long as their shortest ISIs. Perhaps future studies using similarly large differences in ISIs with earthworms might demonstrate an ISI effect on trials to habituation.

Our study further shows that housing worms at 7° C between daily sessions appears to disrupt worms' retention of habituation. Worms housed at room temperature, maintained at 21° C, showed statistically significant drops in mean responses to habituation across the three experimental days, perhaps showing long-term memory formation. Although worms cooled between daily sessions showed declines in mean trials to habituation across experimental days, the declines were not statistically significant. This finding is in harmony with previous reports in an endothermic species (rats) that rapid body cooling immediately after an experimental session interferes with retrieval of the memory for that experimental session (Hinderliter, Webster, & Riccio, 1975; Mactutus & Riccio, 1978). Future research should explore the possibility that "reminder" coolings immediately before daily sessions might improve memory for refrigerated worms, since such reminder coolings enhanced recall among rats in the above-mentioned studies.

That the worms in the 7° C group significantly outperformed worms in the 21° C on the first experimental day is most likely due to chance factors. After all, the first coolings did not occur until after the first experimental session, and worms were randomly assigned to experimental groups by picking out of a box numbers corresponding to their numbered Styrofoam cups. Nor could the refrigerated worms' lack of significant decrease in trials to habituation be due to a "floor" effect, since by the third experimental day the nonrefrigerated worms showed significantly fewer trials to habituation than did the refrigerated worms.

One final aspect of this research goes beyond the theoretical to the practical. Our study shows that simple learning processes can be studied in earthworms for a fraction of the cost and hassle such study would require for vertebrates such as laboratory rats. Indeed, the entire apparatus and worm cost for this study equaled the cost of about two rats. Ongoing pilot studies in our lab are showing that other simple learning processes, such as Pavlovian conditioning, can be studied just as easily and cheaply using earthworms as subjects. Perhaps high school and college lab-based psychology courses could use our apparatus and methodology to save money and avoid many of the concerns associated with using vertebrates in psychological demonstrations and student research (Hull, 1996).

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DIETARY COMPARISON OF *EURYCEA CIRRIGERA* (SOUTHERN TWO-LINED SALAMANDER) LARVAE FROM POND AND STREAM HABITATS IN SOUTHERN WEST VIRGINIA

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ABSTRACT

We give the first dietary report for a lentic population of two-lined salamander larvae (*Eurycea bislineata* complex) and the first dietary comparison of lentic and lotic populations simultaneously. Diets of *Eurycea cirrigera* (Southern Two-lined salamander) larvae were investigated from pond and stream habitats in southern West Virginia during 1994 and 1995. Pond larvae consumed nine prey taxa dominated by chironomid larvae and ostracods, with copepods contributing significantly on most sampling dates. Stream larvae consumed 15 prey taxa dominated by copepods, isopods, and chironomid larvae. Seasonal shifts in diet were apparent at both sites. Comparisons between sites (D = % dietary overlap and r_s = Spearman rank correlation coefficient) indicate that larval diets are different at each site (D = 8.1-41.8; r_s=-0.4091 to 0.5606, p = 0.10-0.96). This is most likely due to differences in prey availability at each site. These results emphasize the generalist nature of Two-lined salamander larvae.

INTRODUCTION

Members of the two-lined salamander complex (*Eurycea bislineata, E. cirrigera,* and *E. wilderae*) are found throughout eastern North America. Larvae are typically found in pools of first-order streams and have a stream-type body shape. They are dusky colored above with six to nine pairs of light dorsolateral spots, and light colored below with numerous iridophores (Petranka, 1998). *Eurycea cirrigera*, the Southern Two-lined salamander, occurs from southern Virginia, west to eastern Illinois and south to northern Florida and eastern Louisiana (Jacobs, 1987; Conant and Collins, 1991). In West Virginia, *E. cirrigera* occupies the southwestern two-thirds of the state in the Allegheny Plateau physiographic province (Jacobs, 1987; Conant and Collins, 1991; Brophy, 1995).

Two-lined salamander larvae are opportunistic generalists that feed on all available prey items in the proper size category. As such, both seasonal and ontogenetic shifts in diet have been documented (Caldwell and Houtcooper, 1973; Burton, 1976; Petranka, 1984; Marcum, 1994; Brophy, 1995). Since Two-lined salamander larvae typically inhabit lotic habitats (Petranka, 1998), all previous reports of diet have come from lotic populations. This is the first dietary report for a lentic population of Two-lined salamander larvae and the first dietary comparison of lentic and lotic populations simultaneously.

MATERIALS AND METHODS

Collections of *E. cirrigera* larvae were made on 10 April 1994 (spring), 11 August 1994 (summer), 30 October 1994 (fall), and 17 January 1995 (winter) from an unnamed pond at the Trump-Lilly Farm near Hinton in Raleigh County, West Virginia, at an elevation of 812 m. Trump-Lilly was an active subsistence farm from the early 1880s to the early 1960s (Nicely et al., 1993), and the pond was most likely constructed as a watering hole for cattle. This small pond (surface area =177 m^2, max. depth = 1.5 m) is located in mesic woods dominated by *Acer* spp., and supports populations of *E. cirrigera, Ambystoma maculatum, Desmognathus ochrophaeus, Notophthalmus viridescens, Rana clamitans*, and *R. sylvatica*.

Collections of *E. cirrigera* larvae were also made on 6 April 1994 (spring), 20 August 1994 (summer), 29 October 1994 (fall), and 14 January 1995 (winter) from Fitzpatrick's Branch, a tributary to Hisey Fork in Wayne County West Virginia, at an elevation of 245 m. Fitzpatrick's Branch is a small intermittent stream that flows through a *Quercus* spp. dominated mixed mesophytic forest. Large rock outcrops line the stream. The streambed contains small pools with a substrate of silt and sand and riffles with coarse pebbles and rocks. This small stream supports populations of *E. cirrigera*, *Desmognathus fuscus*, and *Gyrinophilus porphyriticus*.

Larvae from both sites were captured, euthanized in chloretone, fixed in 10% buffered formalin, and preserved in 70% ethanol. Stomachs were removed and all food items were identified using Merritt and Cummins (1984), Borror et al. (1989), and Peckarsky et al. (1990). Results are presented as the number of each prey item consumed by all larvae and the number of stomachs that contained each prey item.

The degree of dietary overlap between sites is derived from stomach contents using the similarity index:

$$D = [1.0 - 0.5 \sum | p_{x,i} - p_{y,i} |] \times 100$$

Where D is the percentage of overlap and $p_{x,i}$ and $p_{y,i}$ are the proportions of the number of items groups x and y utilized in resource category i (Schoener, 1970; Rathcke, 1976; Holomuzki, 1980). Dietary overlap is calculated between sites during each of four seasons. The same comparisons are made statistically using the Spearman rank correlation coefficient (Snedecor and Cochran, 1989), where rankings are based on the percentage of the number of items found in each prey category.

RESULTS AND DISCUSSION

Larvae from the Trump-Lilly pond consumed nine prey taxa (Table 1). Overall, chironomid larvae and ostracods were the primary prey, but copepods contributed significantly on most sampling dates. Chironomids were most important during January and April, ostracods and chironomids during August, and ostracods alone during October. No larvae had empty stomachs.

Larvae from Fitzpatrick's Branch consumed 15 prey taxa (Table 2). Overall, copepods, isopods, and chironomid larvae were most important. Copepods were most important during January and April, copepods and chironomids during August, and isopods during October. Sixteen percent of larvae collected in October had empty stomachs, while those from all other sampling dates were never empty.

Chironomid larvae, copepods, ostracods, and isopods have all been identified as major prey items in the diet of Two-lined salamander larvae (Caldwell and Houtcooper, 1973; Burton, 1976; Petranka, 1984; Marcum, 1994). In general, chironomids and ostracods are most important in warm weather with copepods and isopods replacing them as weather becomes cooler. Empty stomachs have been reported for all seasons except spring, with the highest occurrences during winter (Caldwell and Houtcooper, 1973; Burton, 1976; Marcum, 1994).

A low degree of dietary overlap consistently occurs between *E. cirrigera* larvae from Trump-Lilly pond and Fitzpatrick's Branch (Table 3). The greatest amount of overlap occurs in spring and summer whereas very little occurs in fall and winter. Statistical comparisons support these findings (Table 3). All correlation coefficients are non-significant ($p = 0.10 - 0.96$), indicating that larval diets are very different at each site.

Two-lined salamander larvae are euryphagic predators (Caldwell and Houtcooper, 1973; Burton, 1976; Petranka, 1984; Marcum, 1994; Brophy, 1995). The low degree of dietary overlap between Fitzpatrick's Branch and Trump-Lilly pond is, therefore, not surprising. Larval diets are most likely different at each site because of differences in prey availability. Even though specific foods differ, comparison of pond and stream populations simultaneously shows that both behave similarly in terms of diet and dietary shift. This novel comparison emphasizes the generalist nature of larvae from the two-lined salamander complex.

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TABLE 1. Prey items of *Eurycea cirrigera* larvae from Trump-Lilly Pond. Values are number of each prey item consumed by all larvae and number of stomachs containing each prey item (in parentheses), n values are number of stomachs examined during each season.

Food Item	Winter (n=25)	Spring (n=21)	Summer (n=25)	Fall (n=26)
Chironomidae larvae	103 (24)	142 (21)	83(24)	107 (21)
Ostracoda	25 (11)	36 (11)	100 (19)	485 (21)
Copepoda	8 (5)	51 (13)	1 (1)	42 (19)
Coleoptera larvae	2 (2)	5 (4)	0 (0)	0 (0)
Odonata nymphs	0 (0)	0 (0)	2 (2)	0 (0)
Annelida	0 (0)	0 (0)	1(1)	0 (0)
Chironomidae pupae	0 (0)	0 (0)	11 (9)	0 (0)
Ephemeroptera nym.	0 (0)	0 (0)	0 (0)	3 (2)
Unidentified	0 (0)	1(1)	0 (0)	3 (3)

TABLE 2. Prey items of *Eurycea cirrigera* larvae from Fitzpatrick's Branch. Values are number of each prey item consumed by all larvae and number of stomachs containing each prey item (in parentheses), n values are number of stomachs examined during each season.

Food Item	Winter (n=25)	Spring (n=22)	Summer (n=25)	Fall (n=25)
Copepoda	880 (24)	506 (22)	49 (17)	5 (5)
Chironomidae larvae	6 (6)	133 (20)	34 (19)	0 (0)
Isopoda	3 (2)	1 (1)	20 (16)	18 (15)
Ostracoda	15 (9)	0 (0)	1 (1)	0 (0)
Annelida	3 (3)	3 (3)	5 (5)	1 (1)
Trichoptera larvae	6 (4)	2 (2)	0 (0)	0 (0)
Tipulidae larvae	1 (1)	0 (0)	2 (2)	0 (0)
Collembola	1 (1)	4 (4)	2 (1)	2 (2)
Plecoptera nymphs	0 (0)	11(9)	0 (0)	0 (0)
Araneae	0 (0)	1 (1)	0 (0)	0 (0)
Hymenoptera	0 (0)	1 (1)	1 (1)	2 (2)
Ephemeroptera nym.	0 (0)	3 (2)	2 (2)	0 (0)
Acari	0 (0)	1 (1)	0 (0)	0 (0)
Amphipoda	0 (0)	0 (0)	2 (2)	2 (2)
Unidentified	2 (2)	1 (1)	2 (2)	0 (0)

TABLE 3. Dietary comparisons of *Eurycea cirrigera* larvae from Fitzpatrick's Branch (FB) and Trump-Lilly Pond (TLP) for each of four seasons. W=winter, SP=spring, SU=summer, F=fall. Comparisons made using percent dietary overlap (D) and Spearman rank correlation coefficient (r_s), n values are number of prey categories compared between sites.

Comparison	% Overlap (D)	r_s	n	Probability
FB-W/TLP-W	8.1	0.5606	10	0.10
FB-SP/TLP-SP	41.8	0.2044	14	0.48
FB-SU/TLP-SU	30.2	-0.0151	13	0.96
FB-F/TLP-F	6.6	-0.4091	10	0.25

BATS OF THE FERNOW EXPERIMENTAL FOREST, TUCKER COUNTY, WEST VIRGINIA

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ABSTRACT

Five species of bats, including the federally endangered Indiana myotis (*Myotis sodalis*), have been documented hibernating in Big Springs Cave on the Fernow Experimental Forest, Tucker County, West Virginia. The present study examined bat activity at the cave entrance and within 2.0km of the cave from April through November 1995. A total of 1,054 bats of nine species were captured; 859 were trapped at the cave entrance, and 195 were captured in mist nets set at six locations. Species captured, in decreasing order of abundance, were: *Myotis lucifugus* (N=363), *M. septentrionalis* (N=307), *Pipistrellus subflavus* (N=237), *M. sodalis* (N=69), *Lasiurus borealis* (N=51), *Eptesicus fuscus* (N=18), *L. cinereus* (N=4), *Lasionycteris noctivagans* (N=4), and *Corynorhinus townsendii virginianus* (N=1). This study produced the first summer records of *M. sodalis* in West Virginia: one in June, five in July, and 10 in August. There was no indication that maternity colonies existed in the immediate vicinity. Over half of the captured bats (N=585) were banded. On 23 February 1996, a bat survey in Big Springs Cave located 990 hibernating bats of four species; 110 were individuals that had been banded on the Forest during the previous summer. Banded bats in the cave represented 11% of the *P. subflavus* banded during the study; 36% of the banded *M. lucifugus*, 86% of the banded *M. sodalis*, and 2% of the banded *M. septentrionalis*.

INTRODUCTION

Caves provide suitable microhabitats for hibernating bats of many species (Twente, 1959; Henshaw, 1972), and caves are abundant geologic features in the limestone strata of West Virginia (Davies, 1958). Because of the importance of caves to bat populations and the relative ease of observing bats during hibernation, most bat studies in West Virginia have focused on hibernating concentrations of these animals (Reese, 1934; Hall, 1972; Davis, 1959; Davis, 1966; Dotson, 1977; Hall and Stihler, 1992; Stihler and Brack, 1992; Delfino, unpub. data). Caves have also been the focus of bat studies during other times of the year (Kretzsch 1961). McKeever (1951) compiled data on the distribution of bats in West Virginia based on both cave and non-cave records, although no areas of the state were surveyed intensively.

The listing of the Indiana bat, *Myotis sodalis*, as a federally endangered species (Fed. Reg. 32:4001) and subsequent recovery efforts drew attention to the habitat needs of this species, including summer habitat away from the hibernacula. Research aided by technological advances, such as those in radio telemetry, have allowed biologists to identify the summer habitats of *M. sodalis* in some portions of the species' range (Brack, 1983; Clark *et al.* 1987; Gardner *et al.*, 1991). A habitat suitability index model for summer habitat needs of *M. sodalis* has been developed (Romme, 1995).

Although concentrations of hibernating *Myotis sodalis*, including one of range-wide significance, have been documented in West Virginia caves (Dotson 1977; Stihler and Brack, 1992; Hall and Stihler, 1992) and suitable summer habitat appears to be present, no summer records of this species have been reported for West Virginia. In Kentucky, Hall (1962) found that at least a few male *M. sodalis* remained in the vicinity of hibernation caves throughout the year and often returned to night-roost in the cave during the summer. The present study attempts to document the presence of *M. sodalis* in West Virginia during the summer by monitoring bats at and near the entrance of a known hibernaculum, Big Springs Cave (Hall, 1972; Stihler, 1995) on the Fernow Experimental Forest, Tucker County. The study also provides data on other bat species, including "tree bats" that are poorly documented because they rarely roost in caves or buildings.

STUDY SITE

The Fernow Experimental Forest, located in Parsons, WV, is an 11,609 ha research forest maintained by the USDA Forest Service, Northeastern Research Station. The area is characterized as having steep slopes covered with hardwood forests. Elevations range from 533 m to 1,112 m. Soils are well-drained medium textured loams and silt loams. Upland oaks are the most common tree species group, and northern red oak (*Quercus rubra*) is most abundant. A belt of Greenbrier Limestone outcrops near Big Springs Gap, and it is in this limestone formation that Big Springs Cave is formed. Mean annual temperature is approximately 8.9° C.

MATERIAL AND METHODS

A 0.91m x 0.91m harp trap was used to capture bats at the entrance of Big Springs Cave. Mist nets (2-ply, 50 denier) were set at six stations within 2.0 km of the cave entrance. Nets were set across potential travel corridors (roads and streams) and over standing water. Bat sampling site locations and descriptions are presented in Table 1. Nets of three lengths (6m, 9m, and 12m) were used. Nets were 2.6m high although two-tier net systems (5.2m high) were constructed (Gardner *et al*., 1989). Both one-tier and two-tier nets were employed. Bats were sampled at least once each month from April to November 1995 (11 capture sessions); sampling effort varied between capture sessions (Table 2).

The following data were recorded for most bats captured: sex, body mass, length of right forearm, time of capture, and capture height (for bats captured in mist nets). Digital balances were used to measure body mass. Observations of parasites were also noted. Several bats were banded (males on the right forearm; females on the left forearm) to obtain information on recapture rates and bat movements between summer habitat and hibernacula. Most bats were banded using yellow plastic bands; numbered (embossed) aluminum bands were used on *Myotis sodalis* and a few *M. septentrionalis*. Bats were released at the point of capture. Hibernating bats in Big Springs Cave were censused on 23 February 1996. Bats were tallied by species, and the species and sex of banded bats were recorded. Statistical analyses were conducted using the JMP statistical package (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Mist netting and harp trapping sessions

During the study, 1,054 bats of nine species were captured (Table 3). *Myotis lucifugus* was the most commonly encountered species (N=363) comprising 34.4% of the captures; *M. septentrionalis* was the second most abundant species (N=307) (Table 3). Most (76.8%) of the bats captured were males (Table 3). Number of captures by species for each trapping session are presented in Table 4. Bats were captured during all capture sessions. Only one bat was captured in late November when there was approximately 0.4 m of snow on the ground outside the cave. This bat, a male *M. septentrionalis*, was captured going into the cave. Over 81% (N=859) of the captures occurred at the cave entrance, but only four species were caught there (Table 5).

Forearm length data for 1,005 bats of nine species and body mass data for 966 bats of nine species are summarized in Table 6. Significant differences in body mass were found between males and females of three species: *Pipistrellus subflavus* ($p<0.01$); *Eptesicus fuscus* ($p<0.05$); and *Lasiurus borealis* ($p<0.01$). Forearm lengths were found to be significantly different between the sexes in two species: *M. septentrionalis* ($p<0.05$) and *L. borealis* ($p<0.05$). Table 7 presents the body mass data for each species by capture session. Species that were captured throughout the study period showed an increase in body mass during the latter portion of the study (September and

October), although the greatest mass was often noted prior to the end of the study (November 1995)(Table 7). This suggests that bats that have acquired sufficient fat reserves enter hibernation earlier than bats that require additional fat reserves.

Over half (N=585) of the bats captured during the study were banded (Table 8). However, only 34 banded bats (5.8%) were recaptured between April and November 1995 (*Pipistrellus subflavus*, N=3; *M. lucifugus*, N=16; *M. septentrionalis*, N=12; and *Lasiurus borealis*, N=3). This suggests that there was a large number of bats in the study area, and that individuals are rarely recaptured.

Summary of Captures by Species

***Pipistrellus subflavus* (eastern pipistrelle):** This species was the third most abundant species captured (N=237)(Table 3). *P. subflavus* were caught at four of the six mist net stations and were present throughout the study period (Table 4). Mean body mass was lowest in late May and highest in mid-September (Table 7). Fleas were noted on two individuals; mites were observed on four specimens.

***Eptesicus fuscus* (big brown bat):** *E. fuscus* were captured at two mist net sites; most were caught during the early portion of the study (May-July) (Table 4). Although none were captured in April, their echolocation calls were detected using ultrasound detectors.

***Myotis lucifugus* (little brown bat):** This was the most commonly captured bat during the study (Table 3). Of the 363 captures, 283 were at the cave entrance (Table 5). This bat was present throughout the study period (Table 4). Average body mass was highest in late September (Table 7). Fleas were observed on 18 individuals; a bat bug (Order Hemiptera: Family Cimicidae) was observed on one individual.

***M. sodalis* (Indiana bat):** Specimens of *M. sodalis* captured in June, July, and August are the first summer records of this federally endangered species in West Virginia (Table 4). Only males were captured in June and July, and all females were captured after 15 August. There was no indication that maternity colonies existed in the immediate vicinity of the study area. *M. sodalis* were captured at the cave entrance and at three of the mist net stations. Male *M. sodalis* probably occur in the study area throughout the year. It is interesting to note, however, that *M. sodalis* were not captured at the entrance of Big Springs Cave in April and May even though they hibernate in the cave. Average mass was lowest in late May and at a maximum in late October (Table 7).

***M. septentrionalis* (northern myotis):** *M. septentrionalis* was the second most commonly encountered bat during the study (N=307)(Table 3); most (N=286) were captured at the cave entrance, but they were also captured at four mist net stations. This is the only species that was captured during every capture session, including late November (Table 4). Mean body weight was lowest in late May and greatest in mid-October (Table 7). Fleas were observed on three individuals; four specimens were infected with mites.

***Lasiurus borealis* (red bat):** A "tree bat," *L. borealis* were captured in mist nets but never at the cave entrance. Most were netted in August and September, but specimens were captured from late June to late October (Table 4). *L. borealis* were netted at four of the mist net stations. Fleas were noted on one specimen.

***L. cinereus* (hoary bat):** Another tree bat, this species was netted at two of the six mist net sites. Three of the four specimens captured were taken in July (Table 4).

***Lasionycteris noctivagans* (silver-haired bat):** Four specimens were netted on 23 October 1995 in a mist net set over a low-water bridge crossing a small stream. All were female, and three of the four were captured during a seven-minute period, the fourth was captured approximately 2.5 hours later. The bats may have been traveling together during migration. Similar concentrations of captures have been observed in Kentucky (John MacGregor, pers. comm.).

***Corynorhinus townsendii virginianus* (Virginia big-eared bat):** This species uses caves in both winter and summer (Barbour and Davis, 1969). This species has never been reported from Big Springs Cave, however, the Arbogast/Cave Hollow cave system, located 8.5 km southeast of Big Springs Cave, harbors both summer and winter populations of this bat. One specimen, a juvenile male, was captured in a mist net set across a small stream below Big Springs Cave on 24 October 1995; a second individual was observed flying over the net at about the same time, but was not captured.

Winter bat survey in Big Springs Cave

The winter bat survey in Big Springs Cave tallied 990 hibernating bats of four species. *Myotis lucifugus* was the most abundant species (N=652), and *M. sodalis* was the second most abundant species (N=183)(Table 8). Ninety-four bats banded on the Fernow Experimental Forest during this study were observed hibernating in the cave and represent 11% of the banded *Pipistrellus subflavus* (N=8), 36% of the banded *M. lucifugus* (N=81), 86% of the banded *M. sodalis* (N=18), and 2% of the banded *M. septentrionalis* (N=3)(Table 8). It appears that most of the *M. sodalis* found near the cave entrance in the summer hibernate in this cave. However, most individuals of other species, especially *M. septentrionalis*, that were trapped at or near the entrance of Big Springs Cave do not appear to hibernate in the cave. No banded bats were observed in other caves surveyed during the winter 1995-96, but a male *M. lucifugus* banded during this study was recovered from Hellhole Cave in Pendleton County, WV (a distance of 38 km) during a February 1997 winter bat survey (Stihler, unpub. data).

Summary

The bat fauna of the Fernow Experimental Forest appears to be diverse, and bats are abundant. Nine of the 13 species of bats documented in West Virginia were captured on the Forest between April and November 1995. The only bat expected to occur in the area that was not observed was *Myotis leibii*; in previous years, this species has been observed hibernating in Big Springs Cave (Stihler, 1995). Although there was no evidence of *M. sodalis* maternity colonies, this species was shown to be present during the summer. Two species of tree bats, *Lasiurus borealis* and *L. cinereus*, appear to be summer residents; *Lasionycteris noctivagans* was observed only during the fall migration period.

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Table 1. Location and description of bat sampling sites on the Fernow Experimental Forest, Tucker County, West Virginia, April - November 1995.

Site Name	Location	Description
Big Springs Cave	4.8 km SSE of city limits of Parsons, WV and 0.45 km NW of the Big Springs Gap trailhead.	Cave is located on a wooded hillside. Harp trap set at entrance of cave. Aspect NNE.
Hickman Slide Hollow	Along Elklick Run approx. 1.7 km N of Big Springs Cave at the junction of USFS Rd. 701 and USFS Rd. 704.	Wooded stream corridor. Mist nets set on low water bridge and over Elklick Run upstream and downstream of the bridge. Aspect N to NW.
Wilson Hollow Weir	Mouth of Wilson Hollow approx. 1.6 km N of Big Springs Cave.	Mist nets set over stream at mouth of hollow above and below the weir. The area is mostly forested with an area of mowed grass near the weir site. Aspect SE.
Below Reservoir	Along Elklick Run below the reservoir dam, approx. 0.9 km NNW of Big Springs Cave.	Mist nets set across Elklick Run and the old road to the dam. The area is wooded. Aspect E.
Reservoir	Upper end of reservoir on Elklick Run, approx. 0.6 km NW of Big Springs Cave.	Mist nets set across upper end of reservoir and across Elklick Run above reservoir. Area around reservoir is forested. Aspect N.
Big Springs Run	Along Big Springs Run approx. 70 m N of Big Springs Cave.	Forested ravine with mist nets set across Big Springs Run near junction with an unnamed tributary. Aspect NW.
Big Springs Gap	Along Big Springs Gap Trail in Otter Creek Wilderness Area, approx. 0.5 km SE of Big Springs Cave.	Mist net set over hiking trail in forested area. Aspect N.

Table 2. Dates and equipment used during eleven bat trapping sessions on the Fernow Experimental Forest, Tucker County, West Virginia, 1995.

Session	Nights Sampled	Number Used		
		HarpTrap	Single-tier Net	Double-tier Net
Late Apr	25-26, 26-27	1	0	0
Late May	23-24, 24-25	1	3	2
Late Jun	27-28, 28-29	1	3	2
Late Jul	25-26, 26-27	1	5	2
Mid Aug	16-17	1	0	0
Late Aug	29-30, 30-31	1	5	2
Mid Sep	13-14	1	0	0
Late Sep	28-29, 29-30	1	4	3
Mid Oct	11-12	1	0	0
Late Oct	23-24, 24-25	1	7	4
Late Nov	26-27	1	0	0

Table 3. Total number of bats captured, by species and sex, during the April - November 1995 bat study on the Fernow Experimental Forest, Tucker County, West Virginia.

Species	Sex			Total	% of All Captures
	Male	Female	Unknown		
Pipistrellus subflavus	184	46	7	237	22.5
Eptesicus fuscus	9	6	3	18	1.7
Myotis lucifugus	265	91	7	363	34.4
Myotis sodalis	64	5	0	69	6.6
Myotis septentrionalis	226	75	6	307	29.1
Lasiurus borealis	34	9	8	51	4.8
Lasiurus cinereus	3	1	0	4	0.4
Lasionycteris noctivagans	0	4	0	4	0.4
Corynorhinus townsendii	1	0	0	1	<0.1
Total	786	237	31	1054	100.0

Table 4. Number of bats captured, by species, during eleven trapping sessions on the Fernow Experimental Forest, Tucker County, West Virginia 1995. P. sub. = *Pipistrellus subflavus*, E. fus. = *Eptesicus fuscus*, M. luc. = *Myotis lucifugus*, M sod. = *M. sodalis*, M. sep. = *M. septentrionalis*, L. bor. = *Lasiurus borealis*, L. cin. = *L. cinereus*, L. noc. = *Lasionycteris noctivagans*, C. tow. = *Corynorhinus townsendii virginianus*.

Session	Species									Total
	P. sub.	E. fus.	M. luc.	M. sod.	M. sep.	L. bor.	L. cin.	L. noc.	C. tow.	
Late Apr	16	0	26	0	9	0	0	0	0	51
Late May	49	2	14	0	5	0	0	0	0	70
Late Jun	1	6	3	1	10	3	0	0	0	24
Late Jul	24	8	34	5	21	6	3	0	0	101
Mid Aug	44	0	20	2	49	0	0	0	0	115
Late Aug	42	2	139	8	93	28	0	0	0	342
Mid Sep	15	0	23	8	36	0	0	0	0	82
Late Sep	39	0	32	16	54	12	1	0	0	154
Mid Oct	3	0	17	16	7	0	0	0	0	43
Late Oct	4	0	25	13	22	2	0	4	1	71
Late Nov	0	0	0	0	1	0	0	0	0	1
Total	237	18	363	69	307	51	4	4	1	1054

Table 5. Bats trapped at the entrance of Big Springs Cave, Fernow Experimental Forest, Tucker County, West Virginia, April - November 1995.

Species	Sex			Total
	Male	Female	Unknown	
Pipistrellus subflavus	175	43	7	225
Myotis lucifugus	205	75	3	283
Myotis sodalis	60	5	0	65
Myotis septentrionalis	212	70	4	286
Total	652	193	14	859

Table 6. Body mass and forearm length data, by species and sex, for nine species of bats captured on the Fernow Experimental Forest, Tucker County, West Virginia, April - November, 1995. Body masses are in grams; forearm lengths are in millimeters. Format is: Mean ± 1 Standard Error (N, Minimum-Maximum). Significant differences between sexes are denoted as * p < 0.05 and ** p< 0.01.

Species	Body Mass		Forearm Length	
	Male	Female	Male	Female
Pipistrellus subflavus	5.71 ± 0.078** (178, 4.1-9.5)	6.35 ± 0.150** (48, 4.8-9.2)	33.8 ± 0.182 (186, 32.0-38.0)	34.4 ± 0.359 (48, 32.0-38.0)
Eptesicus fuscus	17.94 ± 0.449* (8, 16.0-22.2)	19.78 ± 0.243* (8, 19.0-20.7)	45.5 ± 0.423 (8, 44.0-47.0)	46.6 ± 0.532 (8, 44.0-49.0)
Myotis lucifugus	6.84 ± 0.058 (255, 4.8-9.8)	6.85 ± 0.095 (90, 4.8-9.1)	36.6 ± 0.151 (268, 34.0-39.0)	36.6 ± 0.412 (92, 34.0-39.0)
Myotis sodalis	7.58 ± 0.091 (63, 6.1-9.2)	7.32 + 0.471 (5, 6.8-9.2)	37.8 ± 0.156 (64, 34.0-40.0)	38.6 + 0.245 (5, 38.0-39.0)
Myotis septentrionalis	6.53 + 0.081 (222, 4.7-10.0)	6.81 ± 0.130 (75, 5.2-9.8)	35.8 + 0.076* (235, 33.0-39.0)	36.1 ± 0.105* (77, 34.0-38.0)
Lasiurus borealis	9.98 ± 0.178** (36, 8.5-13.1)	13.87 ± 1.269** (8, 9.1-18.4)	39.8 ± 0.269* (36, 36.0-44.0)	41.1 ± 0.459* (8, 39.0-42.0)
Lasiurus cinereus	25.67 ± 1.150 (3, 22.9-28.1)	32.3 (1)	52.7 ± 0.333 (3, 52.0-53.0)	55.0 (1)
Lasionycteris noctivagans	--	12.73 ± 0.448 (4, 11.5-13.6)	--	41.5 ± 0.500 (4, 40.0-42.0)
Corynorhinus townsendii	10.2 (1)	--	45.0 (1)	--

Table 7. Mean body mass and sample size, by species, during eleven trapping sessions on the Fernow Experimental Forest, Tucker County, West Virginia 1995. P. sub. = *Pipistrellus subflavus*, E. fus. = *Eptesicus fuscus*, M. luc. = *Myotis lucifugus*, M. sod. = *M. sodalis*, M. sep. = *M. septentrionalis*, L. bor. = *Lasiurus borealis*, L. cin. =*L. cinereus*, L. noc. = *Lasionycteris noctivagans*, C. tow. = *Corynorhinus townsendii virginianus*.

Species	**Session**										
	Late Apr	Late May	Late Jun	Late Jul	Mid Aug	Late Aug	Mid Sep	Late Sep	Mid Oct	Late Oct	Late Nov
P. sub.	5.48 N=16	4.59 N=47	5.50 N=1	5.54 N=12	5.78 N=42	5.74 N=40	7.34 N=15	6.99 N=38	6.03 N=3	5.73 N=3	--
E. fus.	--	--	18.68 N=5	18.94 N=8	16.00 N=1	--	--	--	--	--	--
M. luc.	6.06 N=26	6.26 N=13	7.30 N=3	6.66 N=21	6.55 N=19	6.73 N=161	7.49 N=22	7.56 N=30	7.32 N=14	7.05 N=25	--
M.sod.	--	--	7.60 N=1	7.46 N=5	7.20 N=2	6.76 N=8	7.75 N=8	7.63 N=16	7.57 N=15	7.91 N=13	--
M. sep.	5.47 N=8	5.30 N=4	6.32 N=7	6.24 N=10	6.26 N=46	6.26 N=92	7.19 N=36	7.58 N=51	7.84 N=7	6.93 N=22	6.70 N=1
L. bor.	--	--	12.35 N=2	10.48 N=6	--	10.68 N=21	--	9.73 N=9	--	13.00 N=2	--
L. cin.	--	--	--	28.80 N=3	--	--	--	22.90 N=1	--	--	--
L. noc.	--	--	--	--	--	--	--	--	--	12.70 N=4	--
C. tow.	--	--	--	--	--	--	--	--	--	10.20 N=1	--

Table 8. Results of the winter bat survey conducted in Big Springs Cave on 23 February 1996 and number of bats, by species, banded on the on the Fernow Experimental Forest, Tucker County, West Virginia, April - November 1995.

Species	Number of Bats Observed in Winter	Number Banded			Band Recoveries in Winter		
		Male	Female	Total	Male	Female	Total
Pipistrellus subflavus	146	50	23	73	8	0	8
Eptesicus fuscus	0	1	0	1			
Myotis lucifugus	652	192	36	228	68	13	81
Myotis sodalis	183	17	4	21	15	3	18
Myotis septentrionalis	9	163	63	226	3	0	3
Lasiurus borealis	0	26	5	31	0	0	0
Lasiurus cinereus	0	1	0	1	0	0	0
Lasionycteris noctivagans	0	0	4	4	0	0	0
Corynorhinus townsendii	0	0	0	0	0	0	0
Total	990	450	135	585	94	16	110

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