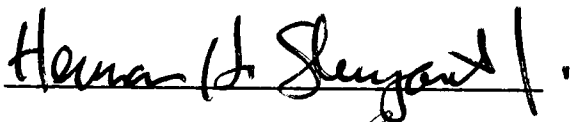


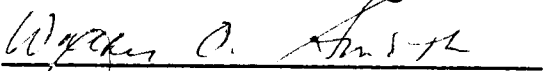
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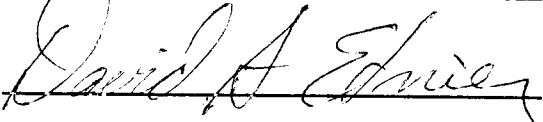
I am submitting herewith a dissertation written by Robert L. Jones entitled "Ecology and Reproductive Biology of Two Species of the *Desmognathus fuscus* Complex (Amphibia: Plethodontidae) in the Southern Appalachians." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology.


Arthur C. Echternacht, Major Professor

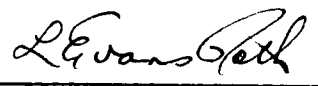
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ECOLOGY AND REPRODUCTIVE BIOLOGY OF TWO SPECIES OF THE
DESMOGNATHUS FUSCUS COMPLEX (AMPHIBIA:PLETHODONTIDAE)
IN THE SOUTHERN APPALACHIANS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Robert L. Jones

June 1982

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ABSTRACT

Morphology, habitat selection, and natural history were compared in two species of Desmognathus from the Unicoi Mountains. Desmognathus santeetlah, a high elevation species restricted primarily to seepages, was smaller in overall size, had relatively shorter rear limbs and tail, and a less distinct dorsal pattern than did the low elevation D. fuscus. Habitats of the two species also differed in some respects, but most of this was due to differences in elevation. Oviposition occurred primarily during July in D. santeetlah and during August in D. fuscus, with mean clutch sizes of 21.33 for the former and 22.89 for the latter. Hatching in the laboratory for both species occurred in 50 to 60 days. The larval period was estimated to be slightly less than one year in both species, and age at maturity between two and three years, with males maturing approximately a year earlier than females. Population densities averaged between $2.76/\text{m}^2$ and $3.27/\text{m}^2$ for D. santeetlah and $1.26/\text{m}^2$ and $1.32/\text{m}^2$ for D. fuscus. Desmognathus santeetlah densities were inversely correlated with the densities of other selected species in its habitats; D. fuscus showed no such correlation. Desmognathus fuscus also exhibited higher tail break frequencies than did D. santeetlah. The differences observed between the two species may have arisen as a result of differential competition, predation, environmental predictability, or a combination of the three.

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I. INTRODUCTION

The family Plethodontidae, the lungless salamanders, is one of the most advanced and the most speciose taxa within the amphibian Order Caudata. The family is thought to have arisen from ambystomatid or preambystomatid ancestral stock during the Mesozoic (Wake, 1966). The Plethodontidae probably evolved in the mountain stream habitats of the southern Appalachians (Wilder and Dunn, 1920; Dunn, 1926), although its present distribution includes much of eastern North America, the western coastal region of the United States, Mexico, Central America, and northern South America. There are also two species in western Europe. The family is composed of two subfamilies, the Plethodontinae and the Desmognathinae; the former is considered to be morphologically the more primitive of the two. The Desmognathinae includes three genera: Phaeognathus, Leurognathus, and Desmognathus, all restricted to eastern North America, generally east of the Mississippi River. Appalachians and Phaeognathus to the Red Hills region of southern Alabama. The genus Desmognathus, which reaches its greatest diversity in the southern Appalachians, presently includes 11 recognized species. Trends in the evolution of this genus have paralleled those in the family, since it includes large stream-dwelling species and progresses toward increasingly smaller body sizes and greater terrestriality. The most widely distributed Desmognathus is D. fuscus, which ranges from southern Canada to Florida and westward to eastern Texas. Recently, a new species, D. santeetlah, was described from

the Great Smoky, Great Balsam, and Unicoi mountains of western North Carolina and eastern Tennessee (Tilley, 1981). This species was described on the basis of both morphological and electrophoretic evidence and is most closely related to D. fuscus. The type locality of D. santeetlah is just below Cherry Log Gap in the headwaters of the Citico Creek watershed in the Unicoi Mountains of Monroe County, Tennessee. In this drainage and in the adjacent North River watershed to the south, D. santeetlah and D. fuscus are apparently ecologically parapatric, with the former occupying springheads and seepage areas near ridgetops and the latter occupying seepages and stream banks at lower elevations. There is apparently no overlap in the distributions of the two species in the Unicoi Mountains nor elsewhere in their ranges, although hybrid populations have been found (Tilley, 1981). The proximity of these two species in both a geographic and an evolutionary sense provides an excellent opportunity to examine how closely related species respond to different sets of environmental conditions. This paper presents the results of a comparative examination of morphology, habitat selection, reproduction, and population structure of these two species in the Unicoi Mountains.

Study Area

The study area was restricted to the northern edge of the Unicoi Mountains along the Tennessee-North Carolina border in roughly a rectangular area defined by the coordinates 35° 17' 30" on the south, 35° 32' 30" on the north, 84° 00' 00" on the east,

and 84° 07' 30" on the west (U.S.G.S. Whiteoak Flats, Tallassee, and Big Junction Quadrangles). This area is drained by two major stream systems; Citico Creek in the north, which flows directly into the Little Tennessee River (Tellico Reservoir), and the North River in the southern part of the study area. The North River is a tributary of the Tellico River, which also drains into the Little Tennessee. Elevations in the area range from approximately 275 to 1650 m. The flora of the Citico Creek watershed was described by Malter (1977). Briefly, some 95-99 percent of the area is forested, with approximately 70 percent of this in hardwoods. Major components of the overstory vegetation include Liriodendron tulipifera, Tsuga canadensis, Acer rubrum, Betula lutea, and various species of Pinus and Quercus. Understory vegetation includes Rhododendron maximum, Leucothoe axillaris, and species of Amelanchier as major components. The study area is entirely within the boundaries of the Cherokee National Forest, and as such is managed for recreational purposes, primarily hunting and fishing, and for timber production. The salamander fauna of this area is relatively diverse, with 16 species in three families present.

The distributions of fuscus and santeetlah in the Unicoi Mountains were illustrated by Tilley (1981). Additional localities in this area plus those indicated by Tilley are shown in Figure 1. The population of fuscoid salamanders at Big Stack Gap was originally thought to be santeetlah, based on morphological evidence (Tilley, 1981); however, subsequent electrophoretic data have indicated that this is a hybrid population (S. G. Tilley, pers. comm.).

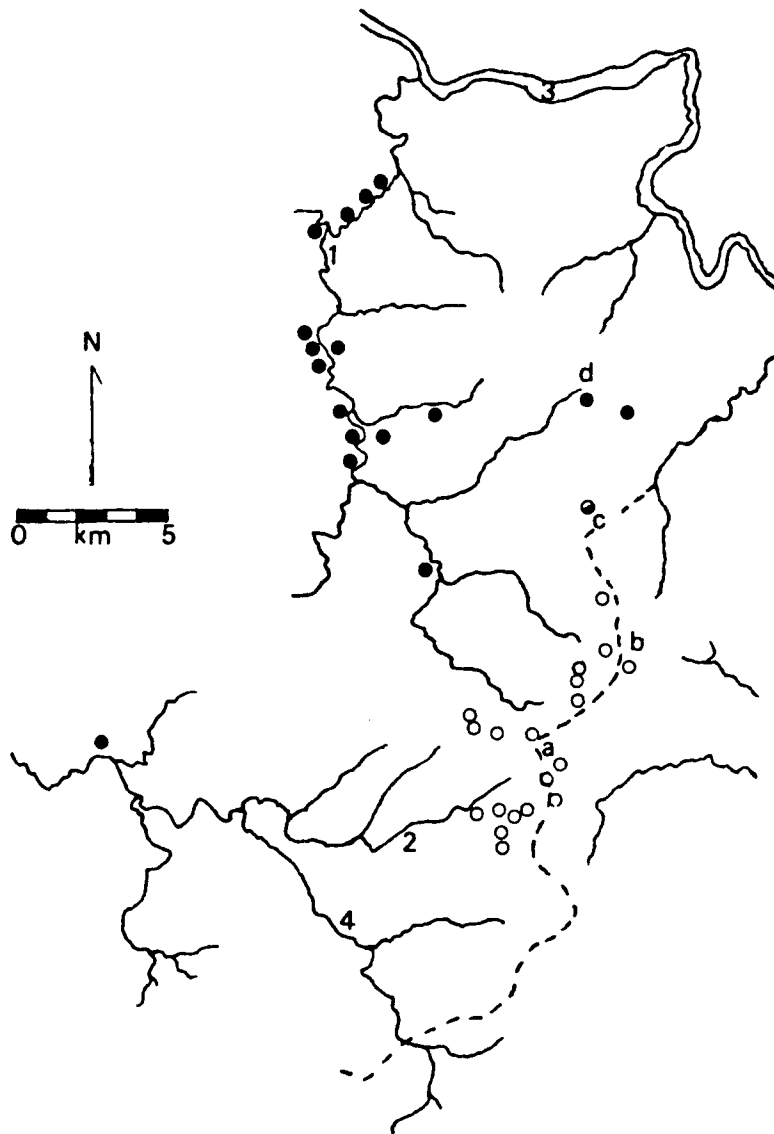


Figure 1. Distribution of *Desmognathus fuscus* and *D. santeetlah* in the study area. Closed circles represent *D. fuscus* localities; open circles, *D. santeetlah* localities; half closed circle, the hybrid population. Numbers represent Citico Creek (1), North River (2), Little Tennessee River (3), and Tellico River (4). Lower case letters represent Beech Gap (a), Cherry Log Gap (b), Big Stack Gap (c), and Farr Gap (d). The dashed line represents the North Carolina-Tennessee border.

II. MORPHOLOGY

Tilley (1981) characterized Desmognathus santeetlah as being similar in general morphology but smaller and slightly more slender than fuscus. When corrected for snout-vent length, santeetlah was found to differ in body proportions, having a smaller trunk width and shorter toes relative to fuscus. Since species of Desmognathus often show extensive interpopulational morphological variation, a detailed analysis of adults and juveniles on both an intra- and interspecific level was conducted.

Tilley (1981) also found differences in dorsal and ventral patterns between these species. Desmognathus santeetlah was described as having a duller and less distinct dorsolateral stripe than fuscus. Ventrolaterally, santeetlah exhibited yellowish pigmentation that fuscus lacked, and had rather more uniformly distributed melanophores than fuscus. Pattern analyses, both dorsally and ventrally, were also performed on the specimens used in the morphological analyses.

Methods

Eight morphological variables (Table 1) were measured to the nearest .1 mm for each individual collected during analyses of population structure and reproductive biology (see below). These specimens were from a majority of the localities indicated in Figure 1. Additionally, each specimen was allocated to one of seven dorsal (Figure 2) and four ventral pattern categories. Ventral

Table 1. Names, mnemonics, and descriptions of the eight morphological variables. All measurements in mm.

Name	Mnemonic	Description
Minimum diameter	MD	Diameter of the smallest hole freshly killed (limp) specimen would fit through on Berol.Rapi-Design Metric Circle template with .5 mm gradations.
Head length	HL	Distance from tip of snout to angle of jaw.
Head width	HW	Head width at angle of jaw.
Head depth	HD	Thickness of head at angle of jaw.
Snout-vent length	SV	Distance from tip of snout to anterior angle of vent.
Trunk length	TR	Distance between insertion of fore and rear limbs measured along venter.
Rear leg length	RL	Length of right rear leg from insertion to tip of longest toe.
Fore leg length	FL	Length of right fore leg from insertion to tip of longest toe.

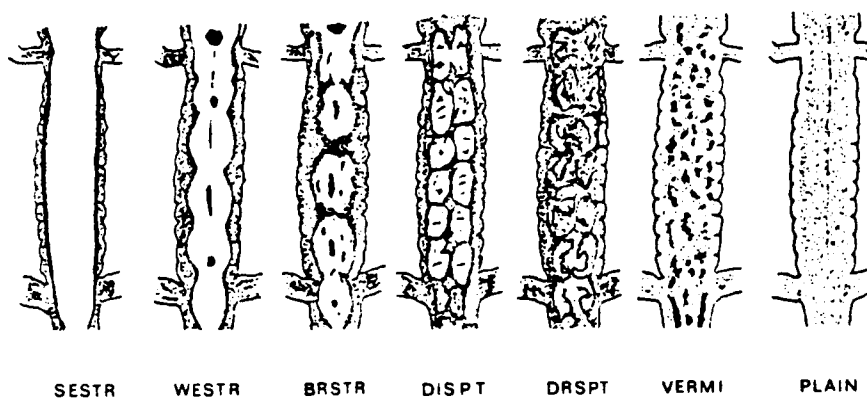


Figure 2. Dorsal pattern categories of Desmognathus fuscus and D. santeetlah. Mnemonics are as follows: SESTR, straight-edged stripe; WESTR, wavy-edged stripe; BRSTR, broken stripe; DISPT, distinct spots; DRSPT, disrupted spots; VERMI, vermiculations; PLAIN, no distinct dorsal pattern.

pattern categories were as follows: no detectable ventral pigmentation, less than 33 percent of ventral surface pigmented, between 33 percent and 67 percent pigmented, and greater than 67 percent of the ventral surface pigmented. These categories were called None, Light, Moderate, and Heavy Pigmentation, respectively. Individuals that were intermediate in pattern type were placed in the category to which they were most similar. Specimens were grouped by sex and maturity class (see below). Morphological variation was analyzed using the SPSS multiple discriminant analysis procedure (Klecka, 1975); pattern variability by G-tests (Sokal and Rohlf, 1969). Five interspecific comparisons were made involving adult males, adult females, juvenile males, juvenile females, and unsexed juveniles. Intraspecific comparisons were between adult males and adult females and between juvenile males and juvenile females.

Results

Interspecific Morphological Comparisons

Descriptive statistics for the variables used in the morphological analyses are shown in Tables 2 and 3. The results of the five interspecific discriminant analyses are shown in Table 4. In each analysis, the test for homogeneity of group covariance matrices (Box's M-test) was significant, indicating that the assumptions underlying the statistical procedure were not precisely met. However, in each case, the test for overall group differences was highly

Table 2. Untransformed means (one standard deviation) of the eight morphological variables for Desmognathus santeetlah. All measurements are in mm.

Variable	Adult		Juvenile		
	Male n=116	Female n=101	Male n=62	Female n=87	Unsexed n=44
Minimum diameter	5.03(0.90)	4.21(0.41)	3.02(0.50)	3.20(0.58)	2.11(0.24)
Head length	6.29(0.91)	5.20(0.47)	4.02(0.69)	4.18(0.67)	2.84(0.53)
Head width	6.27(0.89)	5.26(0.46)	4.13(0.61)	4.21(0.65)	2.98(0.40)
Head depth	3.61(0.64)	3.02(0.24)	2.27(0.45)	2.46(0.52)	1.89(0.32)
Snout-vent length	40.92(5.75)	36.11(2.99)	25.45(4.15)	27.34(4.73)	16.75(2.46)
Trunk length	24.60(3.59)	22.25(2.13)	14.95(2.89)	16.55(3.07)	9.68(1.49)
Rear leg	11.29(1.40)	10.07(0.86)	7.48(1.25)	7.93(1.41)	4.95(0.65)
Fore leg	8.06(1.06)	7.00(0.65)	5.40(1.00)	5.71(0.96)	3.61(0.54)

Table 3. Untransformed means (one standard deviation) of the eight morphological variables for Desmognathus fuscus. All measurements are in mm.

Variable	Adult		Juvenile		
	Male n=131	Female n=103	Male n=41	Female n=75	Unsexed n=60
Minimum diameter	6.19(1.09)	5.41(0.55)	3.90(0.92)	4.07(0.71)	2.34(0.46)
Head length	7.52(1.01)	6.36(0.52)	4.98(0.88)	5.20(0.75)	3.07(0.55)
Head width	7.46(1.02)	6.52(0.56)	5.02(0.88)	5.21(0.87)	3.00(0.71)
Head depth	4.34(0.82)	3.77(0.47)	2.90(0.54)	2.95(0.46)	1.92(0.28)
Snout-vent length	47.53(6.55)	43.80(3.51)	31.39(5.34)	33.53(5.27)	18.33(3.38)
Trunk length	28.40(4.14)	26.84(2.52)	18.93(4.28)	20.33(3.67)	10.42(2.00)
Rear leg	14.77(1.77)	13.65(1.22)	10.15(1.71)	10.84(1.74)	5.83(1.03)
Fore leg	10.15(1.34)	9.28(0.98)	7.17(1.24)	7.55(1.11)	4.37(0.92)

Table 4. Discriminant analysis results from the five interspecific analyses. Mnemonics as in Table 1.

	Adult Male	Adult Female	Juvenile Male	Juvenile Female	Unsexed Juvenile
Eigenvalue	2.468	3.954	1.215	1.470	0.628
Overall F	73.409	96.381	14.280	28.109	14.765
Degrees of freedom	8/238	8/195	8/94	8/153	8/188
Probability	<.001	<.001	<.001	<.001	<.001
Variable					
MD	0.202	-0.325	0.423	0.752	-0.498
HL	0.676	-0.191	0.027	0.152	0.110
HW	0.093	-0.095	-0.040	-0.070	0.558
HD	-0.459	-0.197	0.237	0.220	-0.034
SV	-1.954	0.166	-1.508	-1.011	0.110
TR	-0.197	0.771	-0.538	-1.372	1.059
RL	1.838	-0.986	1.771	1.621	-1.220
FL	0.409	0.165	0.496	0.539	-0.582
Centroids					
<u>D. santeetlah</u>	-1.663	1.998	-0.888	-1.119	0.917
<u>D. fuscus</u>	1.472	-1.959	1.342	1.298	-0.672

significant and estimated on a large number of degrees of freedom (Table 4), indicating that in each case the groups differed geometrically in the multivariate morphological space.

The discriminant functions derived in the analyses of both mature and juvenile males (Table 4) are primarily functions of two variables, snout-vent length (SV) and rear leg length (RL), as indicated by the magnitude of the coefficients associated with them. The opposite signs of the coefficients of these two variables indicate that the relationship between them in differentiating male fuscus from male santeetlah can be expressed as a ratio, since the variables were subjected to a logarithmic transformation prior to the analyses (Green, 1979). The discriminant function in each of these analyses can be interpreted as an axis of morphological variation which at one extreme represents salamanders with short rear legs relative to snout-vent length, and at the opposite extreme represents animals with long rear legs relative to snout-vent length. The value of the centroid of each species (mean of the discriminant scores) indicates the position of the species along the single discriminant function axis (Table 4). The coefficient of the variable RL is positive in each male comparison; that of the variable SV is negative. Animals that have large values for RL relative to small values for SV will have positive discriminant scores, while individuals that have relatively small values for RL and relatively large values for SV will have negative discriminant scores. The centroid of fuscus males is positive in both analyses

(Table 4), indicating that fuscus males have long rear legs relative to snout-vent length. The centroids of santeetlah males are negative, indicating that this species has short rear legs relative to snout-vent length. In other words, when corrected for snout-vent length, male fuscus have relatively longer rear legs than do male santeetlah.

The discriminant functions from the analyses of mature females, juvenile females, and unsexed juveniles (Table 4) are similar to those for males, in that a body length measure relative to a limb length measure is the most important variable in differentiating the two species. However, in these three analyses, trunk length (TR) (rather than snout-vent length) and rear leg length are the most useful for separating the two species. Since these two variables have discriminant coefficients with opposite signs, the relationship between them can again be interpreted as a ratio. The centroids (Table 4) of santeetlah in all three analyses have the same signs as the discriminant coefficients associated with the variables TR and those of fuscus the same signs as the discriminant coefficients associated with RL. Therefore, these analyses indicate that female and unsexed santeetlah have relatively shorter rear legs when corrected for trunk length than do female and unsexed fuscus.

The usefulness of a discriminant analysis in distinguishing between groups can be evaluated by examining the accuracy with which it classifies individuals into the correct groups. Classification results for each discriminant analysis discussed earlier are shown in Table 5. The functions used to classify each individual

Table 5. Classification results from the interspecific discriminant analyses. Numbers and percentages (in parentheses) classified into each group plus the overall misclassification rate for each analysis.

	D. santeetlah	D. fuscus	Misclassification Rate
Adult male			
<u>D. santeetlah</u>	110(94.8)	6(5.2)	5.82%
<u>D. fuscus</u>	10(7.6)	121(92.4)	
Adult female			
<u>D. santeetlah</u>	98(97.0)	3(3.0)	2.44%
<u>D. fuscus</u>	4(3.9)	99(96.1)	
Juvenile male			
<u>D. santeetlah</u>	52(83.9)	10(16.1)	13.14%
<u>D. fuscus</u>	7(17.1)	34(82.9)	
Juvenile female			
<u>D. santeetlah</u>	77(88.5)	10(11.5)	11.31%
<u>D. fuscus</u>	8(10.7)	67(89.3)	
Unsexed juvenile			
<u>D. santeetlah</u>	36(81.8)	8(18.2)	13.14%
<u>D. fuscus</u>	16(26.7)	44(73.3)	

in a particular analysis are formulated using the observations from the original discriminant analysis; therefore, using these classification functions to evaluate the adequacy of a discriminant analysis is somewhat circular. To avoid this circularity, estimated probabilities of misclassification (Kleinbaum and Kupper, 1978) were calculated for each analysis (Table 5). Misclassification rates ranged from approximately 2.5 to 13 percent, with the lowest rates for mature individuals. Mature females are more distinct morphologically than mature males, which Tilley (1981) suggested was a result of lower growth rates in the former after reaching sexual maturity. Juveniles of all classes have approximately a 12 percent average misclassification rate. The higher rates for juveniles is understandable considering that the variables important in the discriminant analyses are reflective primarily of size differences rather than related to differences in shape.

Another morphological variable measured but not included in the discriminant analyses was tail length. This variable was omitted since a relatively high percentage of individuals of each species (see below) did not have complete tails, and using this variable in the analyses would have resulted in greatly reduced sample sizes for some sex and maturity classes. Tail length was defined as the distance from the anterior angle of the vent to the tip of the tail and was measured for each specimen. For those individuals which were judged to have complete tails, descriptive statistics are shown in Table 6. The relationship between tail length and snout-vent

Table 6. Results of regressions of snout-vent length on tail length for Desmognathus fuscus and D. santeetlah of all sexes and maturity classes. F-tests in both cases were significant at .0001 level.

Species	Source	df	SS	MS	F	R ²
<u>D. santeetlah</u>	Model	1	23314.085	23314.085	2404.57	.9014
	Error	263	2549.975	9.696		
	Total	264	25864.060			
<u>D. fuscus</u>	Model	1	48356.072	48356.072	1646.08	.8826
	Error	219	6433.457	29.376		
	Total	220	54789.529			

length for both species is shown in Figure 3. Separate regressions were calculated for each species (Table 6) and subsequently compared using large-sample Z-tests (Kleinbaum and Kupper, 1978). The comparisons indicated that the regression lines had equal intercepts ($Z = 1.27$, $p = .20$), but different slopes ($Z = 5.71$, $p < .001$). These results indicate that juveniles of each species have similar tail lengths relative to snout-vent lengths, but that mature fuscus have relatively longer tails when corrected for snout-vent length than do santeetlah.

Intraspecific Morphological Comparisons

The presence of sexual dimorphism apparently varies among species of Desmognathus. Juterbock (1978) found dimorphism in fuscus and D. welteri in terms of the number of costal folds between adpressed limbs. However, there was no difference in this character between male and female D. monticola. Folkerts (1968) observed that male fuscus were larger in overall size and had relatively longer heads than female fuscus. The results of intraspecific discriminant analyses of morphological variables for both species are shown in Table 7. Adults and juveniles of both sexes of santeetlah can be distinguished primarily on the basis of relative trunk length (TR). The discriminant function derived from the adult male-female santeetlah analysis has its largest coefficient associated with the variable TR. This variable is also the only one other than MD (minimum diameter) to have a negative coefficient. Since the centroid of adult female santeetlah is negative and that of adult

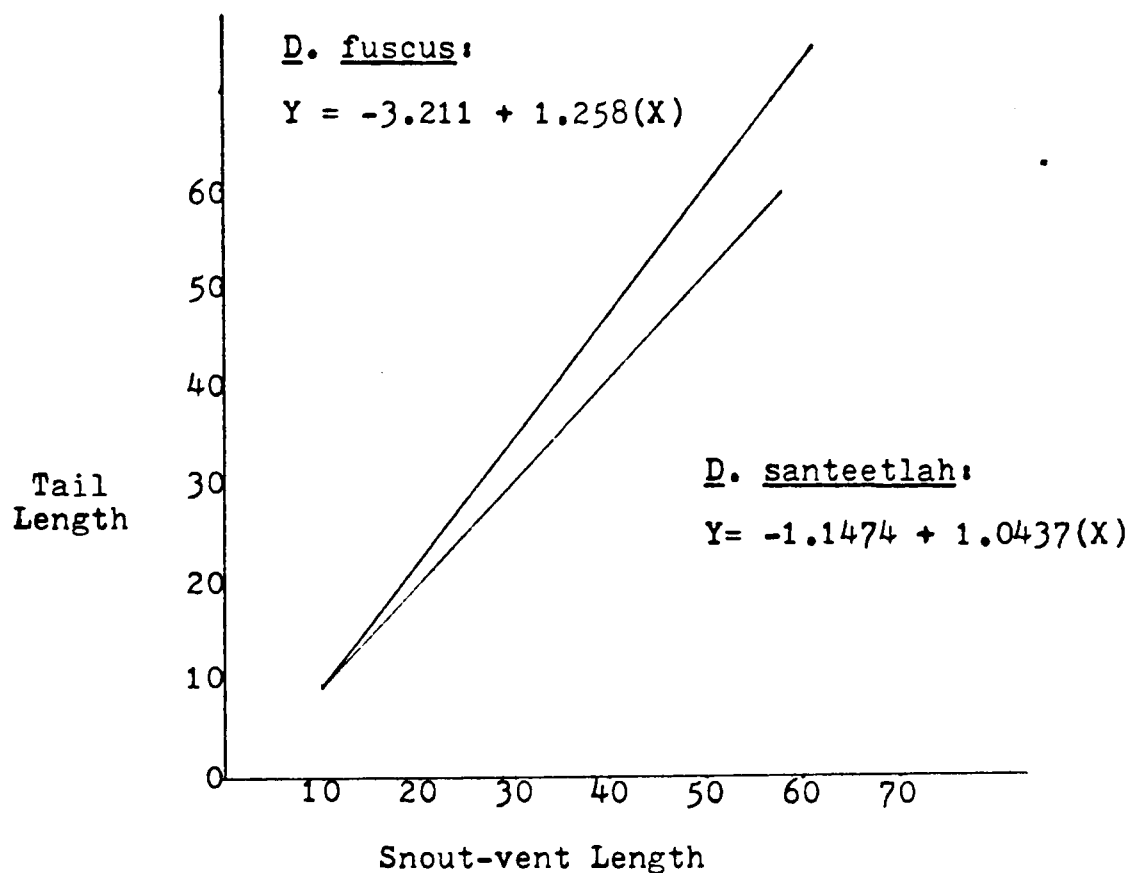


Figure 3. Regressions lines from analyses of snout-vent length on tail length for Desmognathus fuscus and D. santeetlah.

Table 7. Discriminant analysis results from the four intraspecific analyses. Mnemonics as in Table 1 (p. 6).

	D. santeetlah		D. fuscus	
	Adult	Juvenile	Adult	Juvenile
Eigenvalue	0.892	0.154	0.996	0.076
Overall F	23.242	2.708	28.013	1.007
Degrees of freedom	8/208	8/140	8/225	8/107
Probability	<.001	<.01	<.001	.25
Variable				
MD	-0.054	-0.333	-0.049	-0.600
HL	0.720	-0.442	1.600	0.121
HW	0.689	-0.848	-0.035	-0.680
HD	0.296	0.132	-0.132	-0.544
SV	0.483	0.464	0.384	2.050
TR	-1.619		-1.786	0.441
RL	0.138	-0.515	0.352	-0.006
FL	0.134	0.001	0.248	-0.370
Centroids				
Male	0.877	-0.462	0.881	-0.369
Female	-1.007	0.329	-1.121	0.202

males is positive (Table 7), the analysis indicates that adult females have relatively longer trunk lengths than do adult males. The discriminant function of the juvenile male-female santeetlah analysis is similar to that for the adults, with TR as the best discriminating variable. The coefficient associated with this variable is positive, as are those for HD, SV, and FL. The interpretation of this discriminant function is the same as that for adults; i.e., trunk lengths are relatively longer in juvenile females than in juvenile males. The differences in the signs of the coefficients in these two analyses are a result of the generally larger overall sizes of juvenile females relative to juvenile males (Table 2).

The discriminant function from the analysis of adult male and female fuscus indicates that two variables, head length (HL) and trunk length, are the most important in discriminating between the two sexes. Since the coefficients of these variables have opposite signs, the relationship between them, as before, can be interpreted as a ratio. The discriminant function indicates that adult male fuscus can be distinguished from adult female fuscus on the basis of their relatively longer heads and shorter trunks.

The test for homogeneity of covariance matrices for juvenile fuscus was not significant ($F = 1.27$, $df = 36/23364$, $p = .1317$), nor was the overall test of the discriminant analysis ($F = 1.01$, $df = 8/107$, $p < .25$). This indicates that juvenile fuscus do not exhibit sexual dimorphism, at least in terms of the morphological

variables considered here. The discriminant function in this case indicates that juvenile females are, on the average slightly larger than juvenile males, but that this difference is not statistically significant.

The classification results for the intraspecific discriminant analyses are shown in Table 8. Misclassification rates are lowest for adult (approximately 16 percent), which is almost half that of juveniles in each species.

Interspecific Pattern Analyses

The results of the dorsal pattern counts for each species regardless of sex or maturity class are shown in Table 9. Desmognathus santeetlah is more variable in dorsal pattern than fuscus, since no pattern type included greater than approximately 25 percent of the 410 individuals examined. The majority of santeetlah had some form of spotted pattern (43.91 percent; distinct or disrupted spots), followed by striped patterns (33.42 percent) and finally by essentially patternless dorsums (22.69 percent; vermiculations or plain).

Desmognathus fuscus has a more typical dorsal pattern than santeetlah, in that one pattern type (wavy stripe) accounted for over 43 percent of the 410 animals examined. Striped patterns accounted for 73.9 percent of the dorsal patterns observed in this species, while spotted patterns were found in 25.61 percent and less than one half of one percent were patternless. A test for homogeneity of dorsal patterns in these two species was significant ($G = 315.87$, 6 df, $p < .005$).

Table 8. Classification results from the intraspecific discriminant analyses. Numbers and percentages (in parentheses) classified into each group plus the overall misclassification rate for each analysis.

	<u>D. santeetlah</u>		<u>D. fuscus</u>	
	Male	Female	Male	Female
Adult				
Male	87(75.0)	29(25.0)	115(87.8)	16(12.2)
Female	15(14.9)	86(85.1)	17(16.5)	86(83.5)
Misclassification rate	17.36%		15.87%	
Juvenile				
Male	41(66.1)	21(33.9)	26(63.4)	15(36.6)
Female	35(40.2)	52(59.8)	32(42.7)	43(57.3)
Misclassification rate	34.46%		38.97%	

Table 9. Dorsal pattern frequencies for all sex and maturity classes. Mnemonics as in Figure 2 (p. 7). N=410 for both species.

	SESTR	WASTR	BRSTR	DISPT	DRSPT	VERMI	PLAIN
<u>D. santeetlah</u>							
Percent	2 0.5	48 11.7	87 21.2	81 19.8	99 24.1	58 14.1	35 8.5
<u>D. fuscus</u>							
Percent	77 18.8	179 43.7	47 11.5	48 11.7	57 13.9	1 0.2	1 0.2

Ventral pattern results are shown in Table 10. Most santeetlah (>99 percent) has some form of ventral mottling. The category with the highest percentage was that of light mottling (52.44 percent); moderate and heavy mottling were found in approximately equal percentages (22.44 and 24.15 percent, respectively).

Desmognathus fuscus had essentially the same sort of ventral pattern distribution as santeetlah (Table 10), except that a considerably higher percentage had unmarked venters (13.9 percent). The test for homogeneity of ventral patterns between the two species was also found to be significant ($G = 63.1$, 3 df, $p < .001$).

Intraspecific Pattern Analyses

Dorsal pattern counts for sexable juvenile and adult santeetlah are shown in Table 11. Maturity class and dorsal pattern were highly associated ($G = 88.89$, 5 df, $p < .05$). The strong association between maturity class and dorsal pattern is a result of the presence of relatively few juveniles with unmarked dorsums (vermiculations and plain categories) and the presence of relatively few adults with distinct spots. This difference in patterns is a result of a general darkening and loss of pattern in older adults and to the retention of larval spots in juveniles.

Ventral patterns were also examined in santeetlah (Table 12). Tests of independence between maturity class, sex and ventral pattern were similar to those for dorsal pattern, since both maturity class ($G = 64.7$, 2 df, $p < .005$) and sex ($G = 8.264$, 2 df, $p < .005$)

Table 10. Ventral pattern frequencies for all sex and maturity classes in terms of degree of pigmentation. N=410 for both species.

	Degree of Pigmentation			
	Heavy	Moderate	Light	None
<u>D. santeetlah</u>	99	92	215	4
Percent	24.9	22.4	52.4	1.0
<u>D. fuscus</u>	68	98	187	57
Percent	16.6	23.9	45.6	13.9

Table 11. Dorsal pattern frequencies for Desmognathus santeetlah by sex and maturity class. SESTR and WASTR categories were pooled because of zero frequencies in some classes. Mnemonics as in Figure 2 (p. 7).

Sex	Maturity Class	SESTR + WASTR	BRSTR	DISPT	DRSPT	VERMI	PLAIN
Male	Adult	9	33	3	21	27	23
	Percent	7.8	28.4	2.6	18.1	23.3	19.8
	Juvenile	12	10	22	14	2	2
	Percent	19.4	16.1	35.5	22.6	3.2	3.2
Female	Adult	14	28	5	25	21	8
	Percent	13.9	27.7	4.9	24.7	20.8	7.9
	Juvenile	11	14	24	30	6	2
	Percent	12.6	16.1	27.6	34.5	6.9	2.3

Table 12. Ventral pattern frequencies for Desmognathus santeetlah by sex and maturity class. Light and None categories were pooled because of zero frequencies in some classes.

		Maturity Class	Heavy	Moderate	None + Light
Male	Adult		57	27	32
	Percent		49.1	23.3	27.6
	Juvenile		2	10	50
	Percent		3.2	16.1	80.6
Female	Adult		29	32	40
	Percent		28.7	31.7	39.6
	Juvenile		10	22	55
	Percent		11.5	25.3	63.2

were significantly related to ventral pattern. In the first test, adults have generally heavier ventral pigmentation than juveniles. In the second test, males have a higher percentage of heavily pigmented venters relative to those that are moderately pigmented, than do females.

Unsexed juveniles were compared to sexable juveniles and both dorsal ($G = 16.0$, 5 df, $p < .001$) and ventral patterns ($G = 15.32$, 2 df, $p < .001$) were found to be significantly different (Table 13).

Dorsal pattern counts for sexable fuscus are shown in Table 14. In this species, dorsal pattern is independent of sex ($G = 5.01$, 4 df, $.5 < p < .10$) but is strongly related to maturity class ($G = 114.4$, 4 df, $p < .005$), primarily because of large numbers of spotted juveniles.

Table 13. Dorsal and ventral pattern frequencies for sexable and unsexed juvenile Desmognathus santeetlah. SESTR and WASTR, VERMI and PLAIN, and Light and None categories, respectively, were pooled because of zero frequencies in some classes. Mnemonics for dorsal patterns as in Figure 2, p. 7.

	Dorsal Pattern					Ventral Pattern		
	WASTR	BRSTR	DISPT	DRSPT	VERMI + PLAIN	Heavy	Moderate	Light + None
Sexable	23	24	46	44	12	12	32	105
Percent	15.4	16.1	30.9	29.5	8.0	8.0	21.5	70.5
Unsexed	4	2	27	9	2	1	1	42
Percent	9.1	4.5	61.4	20.4	4.5	2.3	2.3	95.4

Table 14. Dorsal pattern frequencies for Desmognathus fuscus by sex and maturity class. VERMI, PLAIN, and DRSPT categories were pooled because of zero frequencies in some classes. Mnemonics as in Figure 2, p. 7.

Sex	Maturity Class	SESTR	WASTR	BRSTR	DISPT	VERMI + PLAIN + DRSPT
Male	Adult	25	63	19	6	18
	Percent	19.1	48.1	14.5	4.6	13.7
	Juvenile	10	14	3	6	8
	Percent	24.4	34.1	7.3	14.6	19.5
Female	Adult	21	47	10	12	13
	Percent	20.4	45.6	9.7	11.6	12.6
	Juvenile	13	25	13	12	12
	Percent	17.3	33.3	17.3	16.0	16.0

Ventral patterns in fuscus (Table 15) were found to be independent of sex ($G = 3.2$, 3 df, $.5 < p < .10$) but were again strongly related to maturity class ($G = 20.72$, 3 df, $p < .005$), with higher relative percentages of juveniles with lightly or unpigmented venters.

Unsexed juveniles and sexable juveniles were also compared (Table 16) and were found to differ in both dorsal ($G = 13.8$, 4 df, $p < .005$) and ventral patterns ($G = 67.14$, 2 df, $p < .005$).

Discussion

To reiterate, fuscus is on the average larger than santeetlah; however, santeetlah is relatively more elongate than fuscus. Morphological differences among adult and juvenile males of the two species are primarily functions of the variables snout-vent length and length of the rear leg. These differences could result from relative differences in snout-vent length, in rear leg length, or in both. Since there is an absolute difference in size between the two species, the most parsimonious explanation is that differences between males are in terms of the length of the rear legs; i.e., male santeetlah have shorter rear legs than do male fuscus when corrected for snout-vent length.

Interspecific morphological differences in females and unsexed juveniles are similar to those among males. In these groups, differences are expressed in terms of trunk length relative to rear leg length. Since trunk length rather than snout-vent length was

Table 15. Ventral pattern frequencies for Desmognathus fuscus by sex and maturity class.

Sex	Maturity Class	Heavy	Moderate	Light	None
Male	Adult	35	37	56	3
	Percent	26.7	28.2	42.7	2.3
	Juvenile	4	7	25	5
	Percent	9.7	17.1	61.0	12.2
Female	Adult	18	38	44	3
	Percent	17.5	36.9	42.7	2.9
	Juvenile	10	16	42	7
	Percent	13.3	21.3	56.0	9.3

Table 16. Dorsal and ventral pattern frequencies for sexable and unsexed juvenile Desmognathus fuscus. PLAIN AND DRSPT dorsal and Heavy and Moderate ventral pattern categories were pooled because of zero frequencies in some classes. Mnemonics for dorsal patterns as in Figure 2, p. 7.

		Dorsal Pattern					Ventral Pattern		
		SESTR	WASTR	BRSTR	DISPT	PLAIN + DRSPT	Heavy + Moderate	Light	None
Sexable	23	39	16	18	20	37	67	12	
Percent	19.8	33.6	13.8	15.5	17.2	31.9	57.8	10.3	
Unsexed	8	20	2	22	8	1	20	39	
Percent	13.3	33.3	3.3	36.7	13.3	1.7	33.3	65.0	

the most important body length measure separating the two species, morphological variation in these groups involves relative differences in both rear leg length, as in males, and in trunk length. In other words, female and unsexed juvenile santeetlah have both relatively shorter rear legs and longer trunks than female and unsexed juvenile fuscus. This implies that proportional differences in morphology between species varies with sex. Evidence to support this hypothesis comes from the intraspecific discriminant analyses. Among adult santeetlah, relative difference in trunk length is the best criterion for separating males from females (Table 6). In adult fuscus, however, head length differences relative to trunk length differences are responsible for the greatest morphological variation between the sexes. In other words, sexual dimorphism is not due to variation in the same morphological characters in these two species. Differences in sexual dimorphism of this nature are a result of interspecific differences in proportion either among the males or among the females. In this case, since differences in head length are important intraspecifically among adult fuscus, but not interspecifically between adult males, interspecific differences in trunk length of females are responsible for the differences in sexual dimorphism observed in these two species. This does not imply that differences in head length do not occur between adult male and female santeetlah. The discriminant function coefficient of the variable HL is the second largest in the intraspecific analysis of adult santeetlah (Table 6), which indicates that differences are secondary in relation to the much greater differences in trunk length. In adult fuscus, trunk length differences are not

as pronounced, so that head length is also important in distinguishing the sexes.

The relatively longer trunks of female santeetlah occur at all ages, since TR is the most important variable in the juvenile intra-specific discriminant analysis as well. No evidence of sexual dimorphism was found, however, in juvenile fuscus, at least in terms of the morphological variables measured.

Why do trunk length differences occur intraspecifically between sexes and interspecifically between females of these two species? Juterbock (1978) suggested that trunk length differences between the sexes were a consequence of differences in gamete sizes, and should be especially evident in those species in which females exhibited a relatively high fecundity. Since female santeetlah produce a relatively greater number of ova (see below) than do female fuscus, greater trunk length in this species may be attributable to its relatively greater fecundity.

The relatively shorter rear legs of santeetlah (or the relatively longer rear legs of fuscus) may be related to characteristics of the habitats of these two species. Folkerts (1968) found relatively shorter legs in some spring and swamp associated populations of fuscus in northern Alabama, and relatively longer legs in populations found near streams with steep gradients. He attributed this variation to differences in locomotor requirements, wherein longer legs were advantageous in habitats in which climbing was necessary (e.g., rocky streams) and shorter legs in those habitats where considerable

burrowing into the substrate took place. It is difficult to judge whether the same sort of selective pressures hypothesized by Folkerts (1968) are responsible for some of the morphological differences between santeetlah and fuscus. Many of the streams and seepage areas occupied by these two species have relatively low gradients. The habitats differ somewhat in substrate composition, however (Table 18). The mean surface rock and gravel composition of fuscus habitats in the study area was approximately 63 percent while santeetlah habitats averaged approximately 42 percent. At the same time, organic material averaged 41 percent in santeetlah and 24 percent in fuscus habitats. This may indicate that shorter rear legs in santeetlah are a result of selection for increased burrowing activity relative to fuscus; however, no overt tendency for burrowing was noticed in santeetlah during this study, except perhaps during nesting (see below).

Folkerts (1968) also found distinct differences in pattern types in the aforementioned populations of Alabama fuscus, with the swamp-spring populations essentially patternless and of dull coloration, and the stream bank forms brightly patterned. He attributed these differences to cryptic substrate matching in the two forms. The situation in the Unicoi Mountains seems to be similar, with the duller, greenish, and less distinctly patterned santeetlah found in areas with a relatively higher percentage of moss and dark rocks, while fuscus occupies habitats with light colored rocks and greater amounts of deciduous leaf litter.

III. HABITAT

As stated earlier, Desmognathus santeetlah and fuscus are ecologically parapatric in the Unicoi Mountains, with the former found in the headwaters of streams and in spring runs near the ridgetops and the latter found along streambanks and in seepage areas in the stream coves. The lowest elevation at which santeetlah has been collected in the study area is at approximately 829 m in the North River watershed. Desmognathus fuscus occurs as high as 841 m near Farr Gap in the Citico Creek watershed but generally occurs at much lower elevations. In both the North River and Citico Creek watersheds are large areas (Figure 1, p. 4) where neither species has been found, even though habitat that appears to be appropriate is present. One possible explanation for the distribution of these two species in the study area is that each has precise habitat requirements which essentially restricts santeetlah to higher elevations and fuscus to lower ones. This might also explain the absence of both species from seemingly adequate habitat at intermediate elevations over large areas in both watersheds. In order to evaluate this hypothesis, structural elements of the habitats occupied by each species and of those habitats at intermediate elevations that appeared to be appropriate were measured. These data were then analyzed with the objectives of quantifying and describing habitat variability within species, between species, and comparing the occupied habitats with those where neither species occurred.

Methods

Thirty habitat plots were randomly selected at localities occupied by each species in the study area (Figure 1, p. 4). No more than two plots were measured at any one site, and when this happened, plots were located at least 10 m apart. Five m sections of the stream or seepage area were marked off, regardless of the stream bed width at that point, and were used as the source of the stream bed characteristics. Bank characteristics were sampled by constructing two semicircular plots with 5 m diameters parallel to and at the edge of the stream or seepage on both of its sides at the same location as the five m strip. Twenty habitat variables were measured for each plot (Table 17). In addition to plots in the habitats of the two species, 25 plots from sites that appeared to be suitable for either fuscus or santeetlah, but which had neither, were sampled and the same 20 habitat variables measured. These "vacant" plots were located in both the Citico Creek and North River watersheds, and were judged suitable on the basis of their visual similarity to those occupied by the two species.

The data for each species were analyzed by principal components analyses using the SAS FACTOR procedure (SAS Institute, 1979) in order to summarize and describe the variability of the structural features of each type of habitat. A principal components analysis essentially reduces a multivariate data set to orthogonal and independent linear combinations of the original variables that account for increasingly smaller proportions of the variation in the data set.

Table 17. Names, mnemonics, descriptions, and units of measure of the 20 habitat variables.

Variable name	Mnemonic	Variable description
1. Width of water	WW	Mean of three measures of open continuous water within the 5 m strip. (m)
2. Width of bed	BW	Same as (1), except including exposed sand and gravel. (m)
3. Substrate rock	PR	Percentage of ocular tube sightings by step-point sampling (James and Shugart, 1971) of stream bed rocks greater than 3 cm in length or width along each of three parallel lines running perpendicular to the direction of water flow within the 5 m strip.
4. Substrate gravel	PG	Same as (3), except for sand and rocks less than 3 cm in length or width.
5. Substrate mud	PM	Same as (3), except for mud.
6. Substrate organic matter	PO	Same as (3), except for logs, leaves, or other organic matter in the stream bed.
7. Water depth	WD	Mean of five measures across stream along each of three parallel lines within the 5 m strip. (cm)
8. Rock surface area	RA	Mean surface area of 10 randomly selected rocks greater than 9 cm ² in area from lines as in (3), measured with respect to area of substrate each covered. (cm ²)
9. Rock volume	RV	Mean volume of rocks in (8). (cm ³)
10. Rock density	RN	Mean number of rocks greater than 3 cm in length or width along lines as in (3).
11. Soil compactibility	SS	Mean of five measures from soil penetrometer from five randomly selected points on both stream bank semicircles. (kg/cm ²)
12. Fallen log density	LB	Total count of fallen logs greater than 5 cm in diameter present in both semicircles.
13. Woody stem density	WN	Same as (12), except for woody stems.
14. Herbaceous stem density	HN	Total count of herbaceous stems from two square plots (1/2 m ²) selected randomly in each semicircle.
15. Evergreen overstory	EO	Step-point sample of evergreen overstory (percent) using ocular tube along baselines of semicircles and along perpendicular line bisecting each.
16. Deciduous overstory	DO	Same as (15), except for deciduous overstory.
17. Absent overstory	AO	Same as (15), except for clear sky.
18. Evergreen understory	EU	Same as (15), except for evergreen understory.
19. Deciduous understory	DU	Same as (15), except for deciduous understory.
20. Absent understory	AU	Same as (15), except for bare ground or rock.

To compare the habitats of each species with one another and with the "vacant" habitats, the raw data matrices for each group were pooled and subjected to a principal components analysis. The resulting principal component scores for each observation were then used as input data for a stepwise multiple discriminant analysis.

All raw data were transformed prior to the statistical analyses by either an arcsin transformation (percentages) or a logarithmic transformation (non-percentages). Since those variables expressed as percentages are perfectly intercorrelated as sets, one variable of each set was deleted (percent rock content of substrate, percent absent overstory, percent absent understory) to avoid singularities in the correlation matrices.

Results

Descriptive statistics of the 20 variables for all three types of habitat are shown in Table 18. The results of the principal components analysis for santeetlah habitats are shown in Table 19, those for fuscus in Table 20. Since there are no statistical tests available for evaluating the significance of individual principal components, a standard technique used is to retain any component with an eigenvalue greater than one and to disregard the rest.

The first six principal components in the analysis of santeetlah habitats have eigenvalues greater than one and account for almost 80 percent of the variability in the data set (Table 19). The first principal component (PC1), which accounts for 24.1 percent of

Table 18. Untransformed means (one standard deviation) and sample sizes of the 20 habitat variables for the three types of habitats. Mnemonics as in Table 17.

Variable	<u>D. santeetlah</u> (N=30)	<u>D. fuscus</u> (N=30)	"Vacant" (N=25)
WW	1.57(1.57)	1.42(1.02)	1.04(0.69)
BW	4.15(2.50)	3.20(1.82)	2.65(1.49)
PR	24.93(11.24)	29.03(19.47)	24.48(17.86)
PG	18.00(10.04)	34.20(15.19)	27.68(16.07)
PM	16.07(15.17)	13.03(18.56)	16.12(23.39)
PO	41.00(13.08)	23.73(13.71)	29.32(14.64)
WD	0.99(0.54)	2.91(1.84)	1.57(1.02)
RA	143.87(146.21)	127.97(100.57)	174.40(107.98)
RV	701.77(1219.18)	617.97(692.82)	900.56(965.90)
SS	0.88(0.40)	1.01(0.45)	1.05(0.42)
RN	13.92(9.08)	13.58(9.80)	10.30(9.99)
LB	6.31(4.69)	3.57(1.94)	6.75(4.99)
WN	7.07(5.20)	9.93(6.68)	13.82(9.80)
HN	44.29(38.77)	33.80(35.80)	35.14(19.95)
EO	27.80(28.00)	11.57(15.71)	26.12(26.80)
DO	65.63(20.64)	74.27(16.51)	66.80(25.27)
AO	21.87(8.68)	14.17(8.93)	7.08(6.41)
EU	17.67(14.41)	18.50(16.02)	30.44(17.65)
DU	44.33(22.93)	39.97(19.71)	27.48(22.35)
AU	37.97(19.75)	40.50(16.94)	42.00(20.73)

Table 19. Results of the principal components analysis for the Desmognathus santeetlah habitats. Mnemonics as in Table 17.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.09	2.97	2.33	1.88	1.22	1.06
Percent variance	24.1	17.5	13.7	11.1	7.2	6.2
Cumulative variance	24.1	41.6	55.3	66.4	73.5	79.7
Variables						
WW	.812	-.123	.155	.315	-.001	.186
BW	.827	.004	-.021	.263	.004	-.101
PG	.270	.522	.308	.425	-.094	-.111
PM	-.730	-.026	-.297	.019	-.093	.242
PO	.396	-.410	.179	-.573	.058	.146
WD	.244	-.470	.360	.546	.009	.268
RA	-.097	-.437	-.676	.357	.150	-.099
RV	-.095	-.502	-.694	.322	.266	-.063
SS	-.361	.432	.063	.331	-.478	-.204
RN	.776	.044	.223	.186	.148	-.314
LB	.197	.698	-.125	-.386	.218	.049
WN	-.204	.158	.407	.139	.720	.342
HN	-.480	-.280	.415	.266	-.273	.221
EO	.541	.384	-.356	-.051	-.361	.468
DO	-.670	.108	.378	.078	.213	-.472
EU	-.317	.704	.036	.432	.128	.302
DU	-.145	-.647	.583	-.211	-.121	.038

Table 20. Results of the principal components analysis for Desmognathus fuscus habitats. Mnemonics as in Table 17.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	4.50	2.83	2.39	2.00	1.16
Percent variance	26.5	16.6	14.0	11.8	6.8
Cumulative variance	26.5	43.1	57.1	68.9	75.7
Variables					
WW	.788	-.055	.425	.146	.230
BW	.737	.078	.149	.026	.172
PG	.560	-.410	-.290	.267	-.177
PM	-.655	.311	-.010	-.079	.626
PO	-.708	.120	-.224	-.357	-.366
WD	.530	-.028	.586	.245	.260
RA	-.137	.434	.816	-.109	-.208
RV	-.325	.461	.680	-.119	-.264
SS	-.519	.278	.242	.478	-.045
RN	.746	-.164	-.067	.076	-.484
LB	.421	.057	-.066	-.754	.151
WN	.380	.466	-.272	-.046	.201
HN	-.367	-.259	.075	.648	.003
EO	.131	.771	-.453	.229	.014
DO	-.174	-.510	.464	-.460	.100
EU	.262	.738	.019	.271	-.060
DU	-.537	-.588	.106	.352	.152

the variation, has high positive loading (coefficients) for the variables WW, BW, and RN, and a high neative loading for the variable PM. This component summarizes variation in the size (width) of the stream or seepage area relative to the composition of its substrate. This principal component can be interpreted as representing variation in stream type among santeetlah habitats, which range from relatively wide streams with rocky substrates to relatively narrow streams with muddy substrates.

The second principal component, which explains 17.5 percent of the total variation, has a high negative loading for the variable DU and high positive loadings for the variables LB and EU. This component is best interpreted as summarizing variation in the type of understory vegetation associated with a sample plot. Most of the logs in the study area have extensive growths of moss, hence log number (LB) and evergreen understory are high correleated, as indicated by both their magnitudes and signs on PC2.

The third principal component, which accounts for 13.7 percent of the variation, has high negative loadings for the variables RA and RV, which are different measures of overall rock dimensions. This component therefore summarizes variation in rock size among santeetlah habitats.

The fourth principal component accounts for 11.1 percent of the total variation in the data set and has a high positive loading for the variable WD and a high negative loading for the variable P0. This component summarizes variation in water depth relative to the amount

of organic matter in the streambed. PC4 is best interpreted as representing variation in the volume of water flowing through santeetlah habitats, since streams with a large volume of water would be expected to have little accumulated organic matter, while those with little flow would have a high percentage of organic matter.

The fifth principal component accounts for 7.2 percent of the variation and has a high positive loading for the variable WN. This component summarizes variation in forest density around a site.

The sixth principal component, which explains 6.2 percent of the variability in the data set, has a high positive loading for the variable D0 and a high negative loading for the variable E0. This component summarizes variation in the type of overstory vegetation associated with santeetlah habitats.

Five principal components from the analysis of fuscus habitats had eigenvalues greater than one and accounted for over 75 percent of the total variation in the data set (Table 20). The first principal component, which accounts for 26.5 percent of the total variation, has high positive loadings for the variables WW, BW, and RN, and high negative loadings for the variables PM and P0. This component, as does the first santeetlah habitat component, summarizes variation in size and substrate composition of streams occupied by fuscus, which range from wide streams with rocky substrates to narrow spring runs with mud and organic matter composing the substrate.

The second principal component, which explains 16.6 percent of the variation, has high positive loadings for the variables E0 and EU.

This component summarizes variation in the evergreeness of the vegetation associated with fuscus habitats.

The third principal component, which account for 14.0 percent of the variation in the data set, has high loadings for the variables RA and RV, the variables that describe overall rock dimensions. As with the third santeetlah component, this habitat component summarizes variation in rock size among fuscus habitats.

The fourth principal component, which account for 11.8 percent of the total variation, has a high negative loading for the variable LB and a high positive loading for the variable HN. This component can be interpreted as summarizing variation in the relative openness of a site, because a high herbaceous stem count and relatively few logs implies that sites have few trees and abundant sunlight, while few herbaceous stems and abundant logs indicates a closed canopy.

The fifth principal component, which accounts for 7.0 percent of the total variation, has a high positive loading for the variable PM. This component represents variation in the relative muddiness of the substrates of fuscus habitats.

The results of the principal components analysis using the pooled data of all three habitat types are shown in Table 21. Six principal components had eigenvalues greater than one and together accounted for approximately 75 percent of the variation in the data set. The first component, which accounted for 25.5 percent of the variation in the pooled data set, was similar to those in the analyses of santeetlah and fuscus habitats. This component has

Table 21. Results of the principal components analysis of all three habitat types combined. Mnemonics as in Table 17.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.33	2.25	2.17	1.77	1.19	1.11
Percent variance	25.5	13.2	12.8	10.4	7.0	6.5
Cumulative variance	25.5	38.7	51.4	61.8	68.8	75.4
Variables						
WW	.592	-.306	.479	.208	.225	.001
BW	.690	-.348	.141	.247	.069	.140
PG	.508	.176	.454	-.208	-.211	.239
PM	-.722	.015	-.142	-.263	.272	-.060
PO	-.322	-.194	-.684	.416	-.102	-.162
WD	.116	.078	.746	-.086	.311	-.399
RA	.601	.611	-.292	-.292	.411	-.043
RV	.510	.622	-.323	.417	-.031	-.313
SS	.082	.367	-.029	.028	.102	.481
RN	.763	-.161	.200	.318	-.183	.128
LB	.343	-.362	-.286	-.242	-.317	.269
WN	.377	.373	-.004	-.345	-.099	-.321
HN	-.291	.428	.057	.387	.257	.476
EO	.503	.382	-.466	-.180	.517	.128
DO	-.526	.336	.391	-.011	-.532	-.020
EU	.357	.477	-.183	-.513	.230	.136
DU	-.529	-.113	.281	.550	.085	.111

high positive loadings for the variables BW and RN and a high negative loading for the variable PM, and therefore summarizes variation in stream type among the habitats.

The second principal component has high positive loadings for the variables RA and RV and accounts for 13.2 percent of the total variation. This component represents variation in rock size among habitats.

The third principal component has a high positive loading for the variable WD and a high negative loading for the variable P0 and accounts for 12.8 percent of the variation in the data set. This component, as in the analysis of santeetlah habitats, represents overall variation in the volume of water flow through the sample plots.

The fourth principal component explains 10.4 percent of the variation and has a high negative loading for the variable EU and a high positive loading for the variable DU. This component represents variation in the type of understory vegetation in the sample plots.

The fifth principal component accounts for 7.0 percent of the variation in the data set and has a high negative loading for the variable DO and a high positive loading for the variable E0. This component summarizes variation in overstory vegetation among the three habitat types.

The last principal component (PC6) accounts for 6.5 percent of the total variation and has high positive loadings for the variables HN and SS. This component explains variation in soil compactability and herbaceous stem number, and can best be interpreted as representing

variation in herbaceous growth among the three types of habitat, since the degree of soil compactedness is probably a result of root matting among herbaceous stems.

The mean of the principal components scores for each habitat type on all six principal components are shown in Table 22. By examining these means and relating their signs to those associated with the coefficients on each principal component (Table 21), variation among all three types of habitats can be described. The first principal component, representing variation in stream type, indicates that santeetlah is found in generally smaller and muddier streams than fuscus, and that the "vacant" habitats are intermediate in this respect. The second principal component, which represents variation in rock dimensions, shows that substrate rock sizes are similar in fuscus and the "vacant" habitats, but smaller in santeetlah habitats. The third principal component, which represents variation in stream flow, indicates that santeetlah habitats have relatively low water flow, fuscus habitats have relatively high flow rates, and that the "vacant" habitats are again intermediate in this respect. The fourth principal component, which represents variation in understory vegetation, shows that santeetlah habitats have greater concentrations of deciduous understory and less evergreen understory than do fuscus habitats, which have relatively less evergreen and more deciduous understory than do the "vacant" habitats. In other words, fuscus habitats are

Table 22. Means of the principal component scores on the six principal components from the combined analysis of habitat data.

Principal component	D. santeetlah	D. fuscus	"Vacant"
1	-.051	.153	.042
2	-.463	.256	.249
3	-.451	.666	-.258
4	.559	-.079	-.576
5	.164	-.028	.014
6	.259	-.341	.099

intermediate on this principal component. The fifth principal component, which represents variation in overstory vegetation, shows that santeetlah habitats have a higher percentage of evergreen overstory relative to deciduous overstory than do fuscus habitats, which have a higher percentage of deciduous overstory relative to evergreen overstory than do the vacant habitats. In other words, the vacant habitats are again intermediate with respect to this principal component. The sixth principal component, which represents variation in herbaceous stem number, indicates that santeetlah habitats have a high relative number of herbaceous stems, "vacant" habitats are intermediate in this regard, and fuscus habitats have a relatively low number of herbaceous stems.

The principal component scores from the pooled analysis of habitat data were subjected to a stepwise discriminant analysis, the results of which are shown in Table 23. Four of the six principal components (2, 3, 4, and 6) entered the analysis and two discriminant functions were derived. The four components represent variation in rock size (PC2), relative water flow (PC3), understory vegetation type (PC4), and number of herbaceous stems (PC6) among the three types of habitat. The first discriminant function accounted for almost 79 percent of the variation in the data set and has the largest coefficient associated with PC3, which represents variation in relative water flow among the habitats. This discriminant function separates santeetlah from fuscus habitats, as evidenced by the relative magnitudes and signs of their centroids on this function (Table 23). The second discriminant function accounts for approximately 21 percent of the total variation in the data set and is a function of PC4, which represents overall variation in understory vegetation. This discriminant function serves to separate the "vacant" habitats from those of the two species. The classification results from this discriminant analysis are shown in Table 24. Almost 70 percent of the 85 sample plots were correctly classified, with the highest incorrect classification rate belonging to the "vacant" habitats. Approximately equal percentages of this group were incorrectly classified as either fuscus or santeetlah habitats.

Since fuscus, santeetlah, and the "vacant" habitats occur along an elevational gradient, the principal component scores of

Table 23. Results of the discriminant analysis on the principal component scores from the combined habitat analysis.

	DF1	DF2
Eigenvalue	.864	.230
Percent variance	79.0	21.0
Cumulative variance	79.0	100.0
Variables		
PC2	.521	-.234
PC3	.745	.575
PC4	-.504	.710
PC6	-.456	-.283
Overall F	66.774	16.647
Degrees of freedom	4/79	4/79
Probability	.0001	.0008
Centroids		
<u>D. santeetlah</u>	-1.183	0.185
<u>D. fuscus</u>	0.946	0.410
"Vacant"	0.285	-0.714

Table 24. Classification results from the discriminant analysis of the principal component scores. Percentages are in parentheses.

Actual Group	Number classified as		
	D. santeetlah	D. fuscus	"Vacant"
<u>D. santeetlah</u>	24(80.0)	1(3.3)	5(16.7)
<u>D. fuscus</u>	4(13.3)	21(70.0)	5(16.7)
"Vacant"	6(24.0)	5(20.0)	14(56.0)

each group from the pooled habitat data set were regressed against elevation in order to test whether differences among the habitats were due to inherent dissimilarities in habitat structure, presumably reflecting differences in habitat selection between fuscus and santeetlah, or to differences primarily attributable to differences in elevation. The results of the multiple regression analysis are shown in Table 25. This analysis indicated that there exists a significant relationship between elevation and habitat variability, as indicated by the overall F value; however, this relationship does not explain a majority of the variability in habitat structure, since the R^2 value of .477 is relatively low. The partial regression statistics (Table 25) indicate that four of the six principle components were significantly related to elevation.

Table 25. Results of the multiple regression analysis of the principal components scores from the habitat analysis on elevation.

Source	df	SS	MS	F	Probability	R^2
Model	6	2.332	0.398	11.84	.0001	.477
Error	78	2.559	0.033			
Total	84	4.891				
Partial Regression Statistics						
PC1	1	0.006		0.20	.6598	
PC2	1	0.299		9.10	.0034	
PC3	1	1.318		40.16	.0001	
PC4	1	0.301		9.17	.0033	
PC5	1	0.041		1.25	.2676	
PC6	1	0.367		11.19	.0013	

Discussion

The individual principal components analyses of fuscus and santeetlah habitats show that variation in the structural elements within the different types of habitat is similar. For example, both the first and the third components in each analysis are essentially expressions of variation in the type of stream (size and substrate composition) and in the size of rocks in the substrate, respectively. Three of the remaining four santeetlah components (2, 5, 6) and two of the remaining three fuscus components (2,4) are expressions of variation in vegetation within their respective habitats while the remaining components in each analysis (PC5 in fuscus; PC4 in santeetlah) are related to variation in different elements of the stream bed.

The pooled principal components analysis indicates that overall variation among the three types of habitat is similar to that within the individual species habitats; i.e., the two most important components (PC1 and PC2) are related to stream type and rock size. The first three principal components deal with characteristics of the streambed, the last three with characteristics of the streambanks.

The mean scores of each type of habitat on the first three principal components show that santeetlah occupies habitats that can be characterized as relatively more seepage-like, since they are, on the average, narrower with muddier substrates and smaller rocks than those occupied by fucus (Table 22). The "vacant" habitats

at intermediate elevations are also intermediate in these characteristics. Desmognathus santeetlah habitats also differ from fuscus habitats in terms of the vegetation associated with them, since they tend to have greater percentages of evergreen overstory and deciduous understory as well as higher percentages of herbaceous stems than do the fuscus habitats. Again, the "vacant" habitats tend to be intermediate in terms of these bank vegetation characteristics (Table 22).

The discriminant analysis also supports the contention that santeetlah habitats are relatively more seepage-like than those of fuscus, since the components with the largest coefficients in the first discriminant function (which separates fuscus from santeetlah habitats; Table 23) describe variation in stream flow through the sample plots. In other words, the greatest difference between the habitats of the two species appears to be a function of relative water flow through their respective habitats, a characteristic that also distinguishes seepage areas from streams. The difference between the habitats of the two species and the "vacant" habitats, on the other hand, is primarily a function of variation in understory vegetation, since PC4, which represents variation in this characteristic, had the largest coefficient on the second discriminant function, which separates the occupied from the vacant habitats.

As stated earlier, four principal components entered the stepwise discriminant analysis (Table 23). These four components were also the components that showed significant partial regression

coefficients in the multiple regression analysis (Table 25). The results of these analyses when taken together indicate that, although differences in the structural elements of all three types of habitat are present, these differences show a significant relationship to differences in elevation. In other words, a large proportion of the differences in these three types of habitat can be attributed to differences in elevation. This implies that *fuscus* and *santeetlah* occupy similar types of habitat, with major differences in these habitats being a function of differences in elevation. It also implies that the "vacant" habitats found between the distributions of these two species are similar to those occupied by the two species, with much of the differences again being attributable to differences in elevation.

IV. REPRODUCTIVE BIOLOGY

The reproductive biology of plethodontid salamanders has been a popular topic of research in recent years (e.g., Bruce, 1972; Danstedt, 1975; Houck, 1977; Juterbock, 1978; Nagel, 1977; Tilley, 1968). Variability in certain aspects of reproduction in the family has been attributed to adaptive diversification related to environmental heterogeneity or to the effects of biotic interactions at both the inter-specific and intraspecific level. This section examines the reproductive biology of Desmognathus santeetlah and fuscus in order to document variation both within and between species. This information may provide insight as to the selective pressures that could have led to the isolation and divergence of the two species. Reproductive variation was studied by collecting monthly samples of both adults and juveniles in order to determine seasonal and size related variation in the condition of the reproductive organs, by making field observations of nest site selection and time of oviposition, and by maintaining field collected clutches of eggs in the laboratory in order to determine the approximate dates and length of time required for hatching.

Methods

Specimens were collected from the sites shown in Figure 1 (p. 4) on an approximately biweekly basis over a period of 18 months. In most cases a site was sampled only once; however, if multiple collections were made, they were from different areas within a site. Collecting was conducted during the daylight hours by turning cover objects

and raking through the substrate. Up to 25 individuals were collected at any one time. A total of 410 santeetlah and 410 fuscus were collected (equal numbers strictly by accident) and represent every month of the year except January, February, and March in the former and January and February in the latter. No collections were made in those months because of the poor condition of roads into the study area during those times and because of the virtual absence of surface activity by salamanders due to inclement weather conditions.

Salamanders were killed in a solution of chlorotone within 24 hours of capture and then fixed in a 10% solution of formalin. After hardening for 24 hours, specimens were measured, soaked in water for an additional 24 hours, and then preserved in 70% ethanol. Morphological measurements (Table 1, p. 6) were made after hardening, except for minimum diameter of the salamander (MD), which was made immediately after the animals were killed but before they were fixed in formalin. All specimens were deposited in The University of Tennessee, Knoxville, Vertebrate Zoology Collection.

All specimens were sexed by dissection and examination of their reproductive organs. Measurements of the reproductive organs were made to the nearest .01 mm with the aid of an ocular micrometer fitted to a dissecting microscope. The reproductive variables measured for males are shown in Table 26 and for females in Table 27. Specimens with undifferentiated germ cords were listed as unsexed juveniles.

Juterbock (1978) stated that the best determinants of sexual maturity in Desmognathus males were the presence of sperm in the vasa deferentia and the presence of a mental gland. He felt that the

Table 26. Names, mnemonics, descriptions, and coding of the reproductive variables measured in male salamanders.

Name	Mnemonic	Variable description
Vas deferens diameter	DD	Diameter of the posterior portion of the right vas deferens. (mm)
Vas deferens shape	DS	Condition of the right vas deferens, coded as either convoluted (3), straight (1) or of intermediate shape (2).
Vas deferens color	DC	Pigmentation of the right vas deferens, coded as either fully pigmented (3), white (1), or intermediate (2).
Testis lobe number	TL	Number of lobes per testis, recorded as largest number of lobes on either testis using the counting procedure outlined by Tilley (1973a).
Sperm content of vasa	SP	Relative amount of sperm in right vas deferens, coded as packed (3), empty (1), or intermediate (2).
Mental gland condition	MG	Presence (1) or absence (0) of mental gland as determined by visual inspection.

Table 27. Names, mnemonics, descriptions, and coding of the reproductive variables measured for female salamanders.

Name	Mnemonic	Variable description
Oviduct diameter	DD	Diameter of the posterior third of the right oviduct. (mm)
Oviduct shape	DS	Condition of the right oviduct, coded as either convoluted (3), straight (1), or intermediate (2).
Degree of yolking	YO	Amount of yolk in ovarian follicles, coded as fully yolked (3), unyolked (1), or of an intermediate condition (2).
Ova size	OS	Mean diameter of six largest ovarian follicles based on three largest from each ovary.
Ova number	ON	Total ovarian follicles in females that had OS values greater than 1.0 mm.

latter was of greater significance because of its importance in courtship activities. Sexual maturity of males in this study was evaluated with respect to both of these characteristics. Since sperm production in salamanders may vary seasonally (e.g., Bruce, 1978), older males (those with more than one lobe per testis) were judged to be mature if they had either sperm in the vasa or a mental gland. Younger males (those with one or fewer lobes per testis) were considered to be sexually mature if they had both of these characteristics.

Juterbock (1978) also listed two characteristics of females that he considered indicative of sexual maturity. These were the presence of yolked ovarian ova and/or large, convoluted oviducts. In this study, females with yolked ova and intermediate or convoluted oviducts were considered to be mature, while those with white, unyolked ova or intermediate yolked and straight oviducts were considered to be immature. Mature females with ova greater than 1.0 mm in diameter were considered to be gravid; i.e., carrying a clutch that would be laid during the next nesting season.

Variation in reproductive characteristics was summarized by means of principal components analyses of reproductive variables for each sex and maturity class of each species. Two factors that could lead to variation in these characteristics are relative age of the animals and season of the year. To evaluate this, principal component scores of each group were regressed against snout-vent length, representing variation in age, and date of collection, representing seasonal variation. The month in which the principal component scores were lowest was considered to represent the beginning of the

reproductive cycle and date of collection was coded to represent this. In mature females, the lowest principal component scores occurred in those collected during July and August for santeetlah and fuscus, respectively. Among juvenile females of both species, lowest scores occurred during April. No overt monthly variation was found in male principal component scores, so the same time scales were used for them. This was done because it seems reasonable to assume that the reproductive cycles of adults and maturation of juveniles occur at approximately the same season in both sexes.

There is a positive correlation between snout-vent length and egg number in most species of Desmognathus. This relationship is present interspecifically as well (Tilley, 1968). Studies of the relationship between snout-vent length and clutch size have indicated that it varies regionally (Tilley, 1968; Danstedt, 1975). Variation among species in terms of clutch volume-body volume ratios occur as well (Tilley, 1968). The relationship between clutch size and snout-vent length and that between clutch volume and body volume were investigated in the two species. Clutch size was estimated from the number of enlarged ovarian follicles per gravid female, since ovarian egg number is approximately equal to clutch size in the genus (Tilley, 1973b). Clutch volume was estimated by multiplying the mean diameter of the largest ova (2.00 mm) by clutch size in gravid females collected during May, June, and July. These three months were chosen because oviposition usually occurs during this period, and mean ovarian egg diameter at this time should be a reasonable estimate of the maximum egg diameter for each species. These diameters were 2.51 mm for

santeetlah ($n = 20$) and 2.65 mm for fuscus ($n = 24$). Body volumes were estimated by considering them to be perfect cylinders wherein trunk length and minimum diameter represented the length and diameter of the cylinder, respectively.

Nest site variation was evaluated by locating brooding females and their clutches along streams and seepages. Nests were located by searching the bed and the banks of these sites for a distance of up to 5 m from open water.

Date of oviposition and length of the incubation period were investigated by collecting clutches and their attending females and maintaining them in the laboratory until hatching took place. Females and clutches were placed in plastic containers with moss and mud from the vicinity of nest site and maintained at approximately 20°C.

Results

Male Reproductive Cycle

A summary for both species of the sizes at which 50% of the males of a given snout-vent length (SVL) first exhibited a given state of a given reproductive characteristic are shown in Table 28. The smallest SVL at which 50 percent of male santeetlah have sperm in the vasa is 29 mm. Fifty percent of those that are 31 mm SVL have a visible mental gland. Corresponding sizes for fuscus males are 34 mm and 36 mm SVL, respectively. Using these as minimum estimates, santeetlah males mature at approximately 30 mm SVL; fuscus males are approximately 5 mm

Table 28. Snout-vent lengths at which 50 percent of males had acquired a given reproductive characteristic.

Characteristic	D. santeetlah	D. fuscus
Convolute vasa	32	36
Pigmented vasa	30	36
Sperm in vasa	30	34
Mental gland	31	36
Testes lobe count of 1.0	29	34

larger when they reach sexual maturity. The smallest mature male santeetlah was 23 mm SVL; the smallest mature male fuscus was 34 mm SVL. The size at which the advanced states of each sexual characteristic are first acquired is similar within species for all characteristic, since 50 percent of the male santeetlah between 29 and 32 mm SVL and 50 percent of the male fuscus between 34 and 36 mm SVL had fully convoluted and pigmented vasa, one lobe per testis, sperm in the vasa, and a mental gland. Juterbock (1978) found that the acquisition of a mental gland lagged slightly behind the presence of sperm in the vasa in three species of Desmognathus, including fuscus. The same relationship in development was observed for the two species in this study, since males generally had sperm in the vasa before a mental gland developed (Table 28).

The results of the principal components analysis of male santeetlah reproductive characteristics are shown in Table 29; those for adult male fuscus are shown in Table 30. Three principal components (PC), accounting for 64 percent of the total variation of the reproductive variables, had eigenvalues greater than one in the analysis of adult male santeetlah. The first component, accounting for 30.6 percent of the variation, had high loadings for the characteristics of the vasa (shape and diameter, Table 29) and for testis lobe number. The second PC, representing 17.3 percent of the variation, had the highest loading for vasa pigmentation (DC), and the third PC, which was responsible for 16.8 percent of the total variation, had its highest loading associated with variation in the condition of the mental gland. The principal components from the analyses of adult male fuscus were not as simple to interpret as were those from the analysis of adult male santeetlah.

Table 29. Results of the principal components analysis of reproductive variables for adult male Desmognathus santeetlah. Mnemonics as in Table 26.

	PC1	PC2	PC3
Eigenvalue	1.83	1.04	1.01
Percent variance	30.6	17.3	16.8
Cumulative variance	30.6	47.9	64.7
Variables			
DD	.853	.022	.079
DS	.682	-.152	.044
DC	.035	.811	-.377
TL	.778	.206	.013
SP	.177	-.531	-.283
MG	-.050	.179	.881

Table 30. Results of the principal components analysis of reproductive variables for adult male Desmognathus fuscus. Mnemonics as in Table 26.

	PC1	PC2	PC3
Eigenvalue	1.98	1.06	1.04
Percent variance	33.0	17.7	17.4
Cumulative variance	33.0	50.7	68.1
Variables			
DD	.689	-.217	-.203
DS	.774	.163	.323
DC	.719	.307	.349
TL	.585	-.098	-.520
SP	-.021	-.594	.681
MG	-.209	.731	.206

In this analysis, as in the former, three principal components with eigenvalues greater than one were derived, and these three accounted for 68.1 percent of the total variation in the data set. The first component, which represented 33 percent of the variation in the data set, had loadings for all of the characteristics of the vasa and for testis lobe number. The second PC, which accounted for 17.7 percent of the variation had high loadings for the variables used to determine sexual maturity, e.g., amount of sperm in the vasa (SP), and the condition of the mental gland (MG). Since these coefficients had opposite signs, PC2 contrasts males with packed vasa and no mental gland with those that had mental glands but no sperm in the vasa. The third component, which accounted for 17.4 percent of the total variation, had high loadings for sperm content and for lobe number, and again the coefficients had opposite signs. As such, this component contrasts males with high lobe numbers and little sperm with those that had fewer lobes but vasa packed with sperm.

The results of the regression analyses using the principal components as dependent variables and date of capture and snout-vent length as independent variables are shown for adult male fuscus in Table 31. The first and third PC were significantly related to the independent variables. Slightly over 34 percent of the variation in PC1 scores was accounted for by the two independent variables. Partial regression statistics (Table 31) indicated that SVL was significantly related to PC1 scores while date of capture was not. The regression model explained only 13.1 percent of the variation in PC3 scores, and again

Table 31. Results of the multiple regression analyses for adult male Desmognathus fuscus with principal component scores as dependent variables and snout-vent length (SVL) and date of collection (DATE) as independent variables.

Source	df	SS	MS	F	Probability	R ²
PC1:						
Model	2	44.43	22.21	33.23	.0001	.342
Error	128	85.57	0.67			
Total	130	130.00				
Partial regression statistics:						
SVL	1	44.42		66.44	.0001	
DATE	1	0.35		0.52	.4717	
PC2:						
Model	1	2.31	1.16	1.16	.3170	.018
Error	128	127.69	0.99			
Total	130	130.00				
PC3:						
Model	2	16.98	8.49	9.62	.0001	.131
Error	128	113.02	0.88			
Total	130	130.00				
Partial regression statistics:						
SVL	1	16.93		19.17	.0001	
DATE	1	0.01		0.01	.9412	

partial regression statistics indicated that only SVL was significantly related to this component.

Only the first PC was significantly related to the joint effects of SVL and date of capture in adult male santeetlah (Table 32). The regression model accounted for 61.06 percent of the variation in this component. Partial regression statistics indicated that each independent variable, when the effects of the other were accounted for, was significantly related to PC1 scores. However, SVL accounted for a much greater percentage of the variation in this component than did date of capture (54.05 vs. 5.05 percent, respectively; Table 32).

The principal components analyses for adult males are similar in that PC1 in both species represents overall variation in maturity, ranging from males with small, slightly convoluted vasa and few testis lobes to males with large, fully convoluted vasa and multiple testis lobation. In santeetlah, variation in vasa pigmentation (PC2) and in development of the mental gland (PC3) are statistically independent of variation in the other reproductive variables. This is because vasa pigmentation apparently develops somewhat earlier, and the mental gland somewhat later, than the other reproductive characteristics (Table 28). In fuscus males, both the second and third PC represent variation in sperm content of the vasa relative to other reproductive variables. In general, these two components indicate that sperm production is more variable than in santeetlah, and that the amount of sperm in the vasa is not directly related to the state of development of the other reproductive characters in adult male fuscus. In both species, variation of the reproductive characteristics is related more to overall

Table 32. Results of the multiple regression analyses for adult male Desmognathus santeetlah with principal component scores as dependent variables and snout-vent length (SVL) and date of collection (DATE) as independent variables.

Source	df	SS	MS	F	Probability	R ²
PC1:						
Model	2	70.22	35.11	88.61	.0001	.611
Error	113	44.78	0.40			
Total	115	115.00				
Partial regression statistics:						
SVL	1	62.68		158.17	.0001	
DATE	1	5.81		14.66	.0002	
PC2:						
Model	2	5.49	2.75	2.83	.0630	.048
Error	113	109.51	0.97			
Total	115	115.00				
PC3:						
Model	2	1.82	0.91	0.91	.4070	.016
Error	113	113.18	1.00			
Total	115	115.00				

size differences, presumably representing differences in age, than to differences in season. In other words, adult males show no overt seasonal variation in the condition of their reproductive organs. This aseasonality of reproductive state of both species is further emphasized by the fact that only two adult fuscus, collected in August and October, and no adult santeetlah, lacked sperm in the vasa (Table 33). Courtship behavior in fuscus has been noted in both the fall and the spring (Organ, 1961). Organ (1961) stated that courtship probably did not occur in either late summer (July and August) nor during the winter, the latter because of low temperatures which restricted activity. The best evidence for courtship activity other than direct observation is the presence of spermatophores in the vents of either males or females. In this study, no recognizable spermatophores were found in the vents of either sex, probably as a result of the effects of either the fixative or the preservative on spermatophore structure. However, Juterbock (1978) stated that the diameter of the vasa in male Desmognathus varied seasonally, reaching a maximum during the courtship season. Both the percentage of males with packed vasa and the mean diameter of the vasa approach a minimum during mid to late summer (Table 33), which may indicate a reduction of courtship activity in both species.

The results of the principal components analyses for juvenile males are shown in Table 34. In fuscus, two components had eigenvalues greater than one and accounted for a total of 81.9 percent of the variation in the data set. The first explained 62.8 percent of the variation and had high loadings for all reproductive characteristics except mental gland

Table 33. Frequency of sperm abundance categories and vas diameters by month for adult males of Desmognathus fuscus and D. santeetlah.

	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
<u>D. fuscus</u>										
Vasa sperm	100	100	89	100	75	87	100	94	100	100
Packed	0	0	11	0	21	13	0	0	0	0
Intermediate	0	0	0	0	4	0	-	6	0	0
Absent										
Vasa diameter, mm										
$\bar{x}$	.44	.54	.55	.41	.35	.42	.38	.44	.47	.35
n	8	11	9	21	24	15	12	17	8	6
<u>D. santeetlah</u>										
Vasa sperm										
Packed	100	100	100	95	94	93	82	93	100	100
Intermediate	0	0	0	5	6	7	18	7	0	0
Absent	0	0	0	0	0	0	0	0	0	0
Vasa diameter, mm										
$\bar{x}$	.42	.40	.40	.45	.35	.31	.33	.40	.35	.38
n	8	14	14	20	17	18	11	14	6	12

Table 34. Results of the principal components analyses of reproductive variables for juvenile males of Desmognathus fuscus and D. santeetlah. Mnemonics as in Table 26, p. 54.

	<u>D. santeetlah</u>		<u>D. fuscus</u>	
	<u>PC1</u>	<u>PC2</u>	<u>PC1</u>	<u>PC2</u>
Eigenvalue	3.65	1.08	3.77	1.15
Percent variance	60.8	18.0	62.8	19.1
Cumulative variance	60.8	78.8	62.8	81.9
Variables				
DD	.800	-.018	.820	-.048
DS	.851	.147	.850	.178
DC	.829	.222	.767	-.343
TL	.872	-.271	.943	.204
SP	.890	-.290	.941	-.148
MG	.217	.924	.100	.965

condition. The second, which accounted for 19.1 percent of the variation, had a high loading for mental gland condition. Thus, PC1 summarizes overall reproductive variation, while PC2 summarizes variation in the development of the mental gland that is independent of variation in the other reproductive variables. The analysis of juvenile santeetlah was similar to that for fuscus, since two principal components with eigenvalues greater than one were derived and the interpretation of these components is precisely the same as that for the juvenile male fuscus.

The results of the regression analyses for juvenile male fuscus are shown in Table 35 and for juvenile male santeetlah in Table 36. For each species, only the first component was significantly related to the combined effects of date of capture and snout-vent length. In fuscus, the regression model explained 43.8 percent of the variation in PC1. Partial regression statistics indicated that each independent variable, when the effects of the other were accounted for, was significantly related to PC1 scores. This is in part a result of the high correlation between the two independent variables ($r = .44$, $t = 2.719$, $p < .05$). The regression model for santeetlah accounted for 46.2 percent of the variation in PC1. In this case, snout-vent length, when considered alone, was significantly related to PC1 scores, while date of collection was not (Table 36).

As with the adults, variation in the reproductive characteristics of juvenile males is similar in the two species. In both, the first PC derived summarized overall variation in maturity, while the second PC represented variation in the development of the mental gland. This may reflect the relatively later development of the mental gland

Table 35. Results of the multiple regression analyses for juvenile male Desmognathus fuscus with principal component scores as dependent variables and snout-vent length (SVL) and date of collection (DATE) as independent variables.

Source	df	SS	MS	F	Probability	R ²
PC1:						
Model	2	17.53	8.76	14.82	.0001	.438
Error	38	22.47	0.59			
Total	40	40.00				
Partial regression statistics:						
SVL	1	5.37		9.08	.0046	
DATE	1	4.42		7.47	.0095	
PC2:						
Model	2	3.64	1.82	1.90	.1633	.091
Error	38	36.36	0.96			
Total	40	40.00				

Table 36. Results of the multiple regression analyses for juvenile male Desmognathus santeetlah using principal component scores as dependent variables and snout-vent length (SVL) and date of capture (DATE) as independent variables.

Source	df	SS	MS	F	Probability	R ²
PC1:						
Model	2	28.16	14.08	25.29	.0001	.462
Error	59	32.84	0.56			
Total	61	61.00				
Partial regression statistics:						
SVL	1	26.16		46.99	.0001	
DATE	1	0.04		0.08	.7812	
PC2:						
Model	2	2.25	1.12	1.13	.3300	.037
Error	59	58.75	0.99			
Total	61	61.00				

relative to the other reproductive characteristics, as suggested earlier. Most of the variation in the reproductive variables of juvenile males is a function of variation in size rather than of season. The relatively high correlation between PCl, snout-vent length, and date of collection in juvenile male fuscus results from the fact that maturing males, i.e., those with an intermediate vasa shape and pigmentation, one testis lobe, and either sperm in the vasa or a mental gland, were collected only from late August through December. Maturing santeetlah, on the other hand, were found in all months in which collections were made. This could result from sampling error, or it could indicate that juvenile male fuscus mature primarily during the last half of the year.

Female Reproductive Cycle

Reproductive characteristics. Fifty percent of the female santeetlah which were 32 mm SVL had some degree of convolution of the oviducts; 50 percent of those which were 34 mm SVL has some indication of yolking of the ova. In fuscus females, 50 percent of those which were 41 mm SVL had convolutions of the oviducts, while 50 percent of those which were 37 mm SVL had some degree of yolking of the ova. When these two variables were considered together, 50 percent of the santeetlah females that were 33mm SVL were mature, while 50 percent of the fuscus females that were 39mm SVL were mature. Therefore, females of both species become sexually mature at a size that is approximately 3-4 mm larger than the size at which males reach sexual maturity.

The results of the principal components analyses for adult females of both species are shown in Table 37. Ova number (ON) was not included in these analyses since it was only recorded when ova diameters exceeded 1.0 mm. Each of the analyses resulted in one PC with an eigenvalue greater than one. In fuscus, this component accounted for 72.2 percent of the variation in the data set; in santeetlah, the single PC accounted for 67.9 percent of the total variation. In both analyses, all variables had high positive loadings, indicating that all were strongly correlated.

The results of the multiple regression analyses for adult females of both species are shown in Table 38. In fuscus, the regression model accounted for 36.84 percent of the variation in PC1. Partial regression statistics indicated that date of collection was significantly related to PC1 scores when the effects of size were accounted for, but that the converse was not true. In santeetlah, the regression model accounted for a smaller percentage of the variation in PC1 (20.96 percent). In this case, each independent variable, when the effects of the other were held constant, was significantly related to PC1 scores. However, date of collection accounted for a higher percentage of the variation in PC1 than did SVL (14.65 vs. 4.64 percent).

These analyses indicate that variation in the reproductive characters of adult females of both species is seasonal rather than related to variation in size. This is in contrast to what occurs in males. Moreover, there appears to be greater regularity in the seasonal variation of reproductive characteristics in fuscus than in santeetlah, since the regression model accounted for a larger proportion of the variation in the principal component in the former.

Table 37. Results of the principal components analyses of reproductive variables for adult females of Desmognathus fuscus and D. santeetlah. Mnemonics as in Table 27 (p. 55).

	<u>D. santeetlah</u> PC1	<u>D. fuscus</u> PC1
Eigenvalue	2.72	2.89
Percent variance	67.9	72.2
cumulative variance	67.9	72.2
Variables		
DD	.876	.889
DS	.776	.799
YO	.736	.752
OS	.898	.947

TABLE 38. Results of the multiple regression analyses for adult females of Desmognathus fuscus and D. santeetlah using the single principal component as the dependent variable and snout-vent length (SVL) and date of collection (DATE) as independent variables.

Source	df	SS	MS	F	Probability	R ²
<u>D. fuscus</u>						
Model	2	37.57	18.79	29.16	.0001	.368
Error	100	64.42	0.64			
Total	102	102.00				
Partial regression statistics:						
SVL	1	0.72		1.11	.2941	
DATE	1	36.97		57.38	.0001	
<u>D. santeetlah</u>						
Model	2	20.96	10.48	12.99	.0001	.210
Error	98	79.04	0.81			
Total	100	100.00				
Partial regression statistics:						
SVL	1	4.64		5.76	.0183	
DATE	1	14.66		18.16	.0001	

The principal components analysis for juvenile female santeetlah (Table 39) resulted in two components with eigenvalues greater than one. Together they explained 83.4 percent of the total variation in the data set. The first component had high loadings for all variables except oviduct shape and accounted for 57.6 percent of the variation in the data set. The second component explained 25.9 percent of the variation and had a high loading for oviduct shape. This analysis indicates that, as in adult females, most of the reproductive variables are positively correlated. However, in juvenile santeetlah, variation in oviduct shape is apparently independent of that of the other reproductive variables.

The results of the principal components analysis for juvenile female fuscus are also shown in Table 39. The variable DS (oviduct shape) was not included in the analysis since it did not vary in the 75 individuals examined. One principal component, accounting for 70.4 percent of the total variation, had an eigenvalue greater than one. This component, as in adult females, had high positive loadings for all the variables in the analysis, and thus represents overall variation in maturity among juvenile female fuscus.

The multiple regression statistics for juvenile females of both species are shown in Table 40. In fuscus, the regression model accounted for 47.3 percent of the variation in PC1. Partial regression statistics indicated that variation in this component was related to size rather than to time of collection. The multiple regression results for santeetlah are essentially the same as those for fuscus. Only the first PC of the two derived was significantly related to the

Table 39. Results of the principal components analyses of reproductive variables for juvenile females of Desmognathus fuscus and D. santeetlah. Mnemonics as in Table 27 (p. 55).

	<u>D. fuscus</u> PC1	<u>D. santeetlah</u> PC1	PC2
Eigenvalue	2.11	2.30	1.04
Percent variance	70.4	57.6	25.9
Cumulative variance	70.4	57.6	83.4
Variables			
DD	.775	.886	.118
YO	.850	.766	-.404
OS	.888	.919	-.073
DS		.294	.923

Table 40. Results of the regression analyses for juvenile females of Desmognathus fuscus and D. santeetlah using principal component scores as dependent variables and snout-vent length (SVL) and date of collection (DATE) as independent variables.

Source	df	SS	MS	F	Probability	R ²
<u>D. fuscus</u>						
Model	2	35.00	17.50	32.30	.0001	.473
Error	72	39.00	0.54			
Total	74	74.00				
Partial regression statistics:						
SVL	1	27.68		51.09	.0001	
DATE	1	2.24		4.14	.0456	
<u>D. santeetlah</u>						
Model	2	36.62	18.31	31.14	.0001	.426
Error	84	49.38	0.59			
Total	86	86.00				
Partial regression statistics:						
SVL	1	34.36		58.44	.0001	
DATE	1	1.07		1.82	.1806	
PC2:						
Model	2	2.40	1.20	1.20	.3045	.028
Error	84	83.60	1.00			
Total	86	86.00				

combined effects of the two independent variables. In this case, 46.2 percent of the variation in PC1 was explained by the regression model. Partial regression statistics indicated that most of the variation in PC1 scores was related to variation in size rather than to variation in time of collection.

Clutch size-clutch volume relationships. The mean clutch size for 73 gravid fuscus was 22.89 (range 8-37), which is within the range of mean values reported for this species in other studies (Folkerts, 1968; Danstedt, 1975; Hall, 1977). For 84 gravid santeetlah, mean clutch size was 21.33 (range 10-35). Plots of clutch size versus snout-vent length indicated that the two variables were positively correlated, so they were subjected to least squares linear regression analyses. The raw data were curvilinear, so both variables were transformed to their natural logarithms prior to the analyses. The variation in clutch size explained by variation in SVL was relatively small in both species (Table 41), although fuscus exceeded santeetlah in this respect. The slopes and intercepts of the two regression lines (Figure 4) were compared using both a dummy regression model and an analysis of covariance (Kleinbaum and Kupper, 1978). The dummy regression indicated that the slopes of the two lines were not significantly different ($F = .193$, $df = 1/153$, $p > .25$), but that the intercepts were ($F = 11.94$, $df = 2/153$, $p < .001$). The adjusted mean clutch sizes (adjusted for regression), after transformation back to raw form, were 23.88 for santeetlah and 18.95 for fuscus. These adjusted means were significantly different at the .1 percent level, ($F = 23.94$, $df = 1/154$). These results indicate that for a given snout-vent

Table 41. Results of the clutch size-snout-vent length regressions for Desmognathus fuscus and D. santeetlah.

Source	df	SS	MS	F	Probability	R ²
<u>D. santeetlah</u>						
Model	1	1.414	1.414	35.28	.0001	.301
Error	82	3.288	0.040			
Total	83	4.702				
<u>D. fuscus</u>						
Model	1	1.511	1.511	38.30	.0001	.350
Error	71	2.801	0.039			
Total	72	4.312				

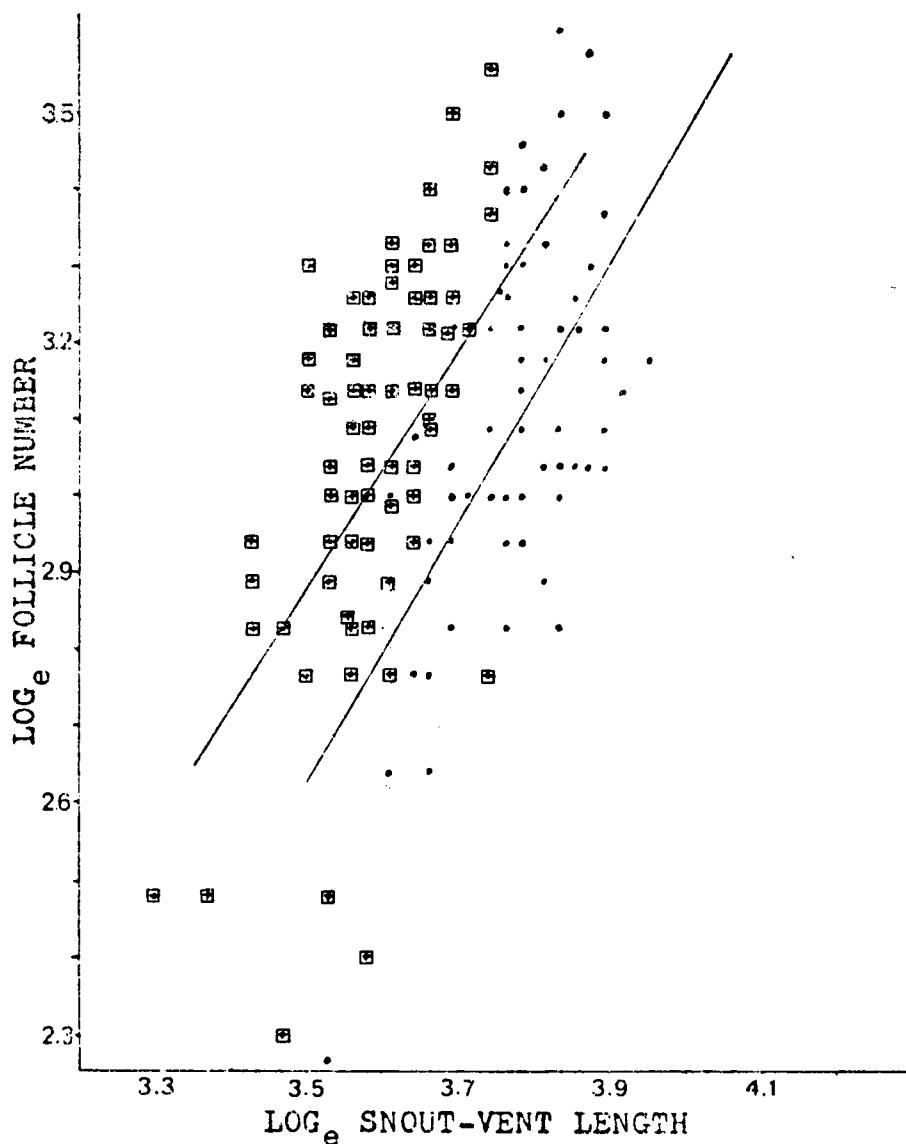


Figure 4. The relationship between body size and fecundity in gravid females of *Desmognathus fuscus* and *D. santeetlah*. Circles represent *D. fuscus*, squares *D. santeetlah*. Regression equations are $\log Y = -2.54 + 1.55(\log_e X)$ for *D. santeetlah* (upper line) and $\log Y = -3.39 + 1.72(\log_e X)$ for *D. fuscus* (lower line), where X is snout-vent length and Y is follicle number.

length, santeetlah produces a significantly greater number of eggs than fuscus. However, the increase in ova produced per unit increase in snout-vent length is identical in the two species.

The mean clutch volume-body volume ratios for santeetlah and fuscus were .579 and .373, respectively. Twice the standard errors on each side of the means did not overlap (Figure 5), which indicates that the means differ at approximately 5 percent level. This indicates that santeetlah produces a significantly greater volume of yolk in relation to its body volume than fuscus.

Dates of oviposition, length of brooding, and nest sites. Evidence for time of oviposition comes from direct observations of nesting in the field and indirectly from comparisons of maximum ova sizes through time. The latter assumes that maximum size of the ovarian follicles occurs just prior to oviposition.

Maximum ova diameters in fuscus were present during the second week in July; minimum diameters were observed during the last two weeks of September (Figure 6). The three females with relatively large ova on 19 September could represent either individuals that were not able to oviposit during the summer, or individuals that were on a different oviposition schedule than the majority of females in the study area. The earliest date for fuscus clutches in the field was 17 July. Two females were discovered in the process of laying on 23 and 29 July. The earliest hatching date for a clutch maintained in the laboratory was 18 August. This may indicate, given the estimate for length of time required for hatching (see below) that

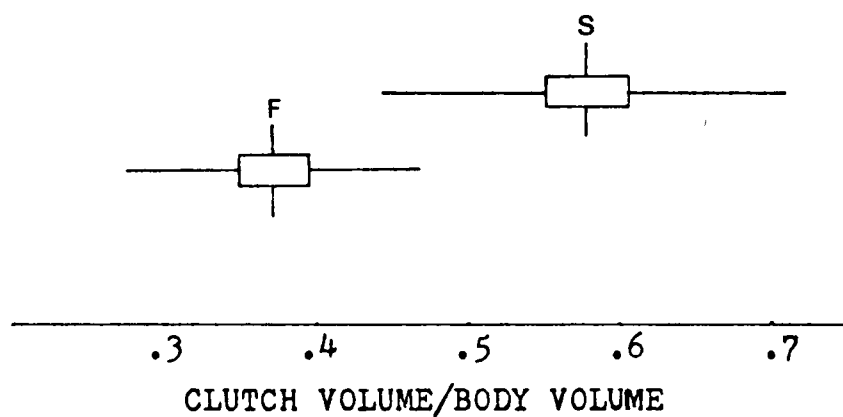


Figure 5. Comparison of clutch volume-body volume ratios. Hollow boxes represent twice the standard error, lines one standard deviation on either side of the mean. F = Desmognathus fuscus; S = D. santeetlah.

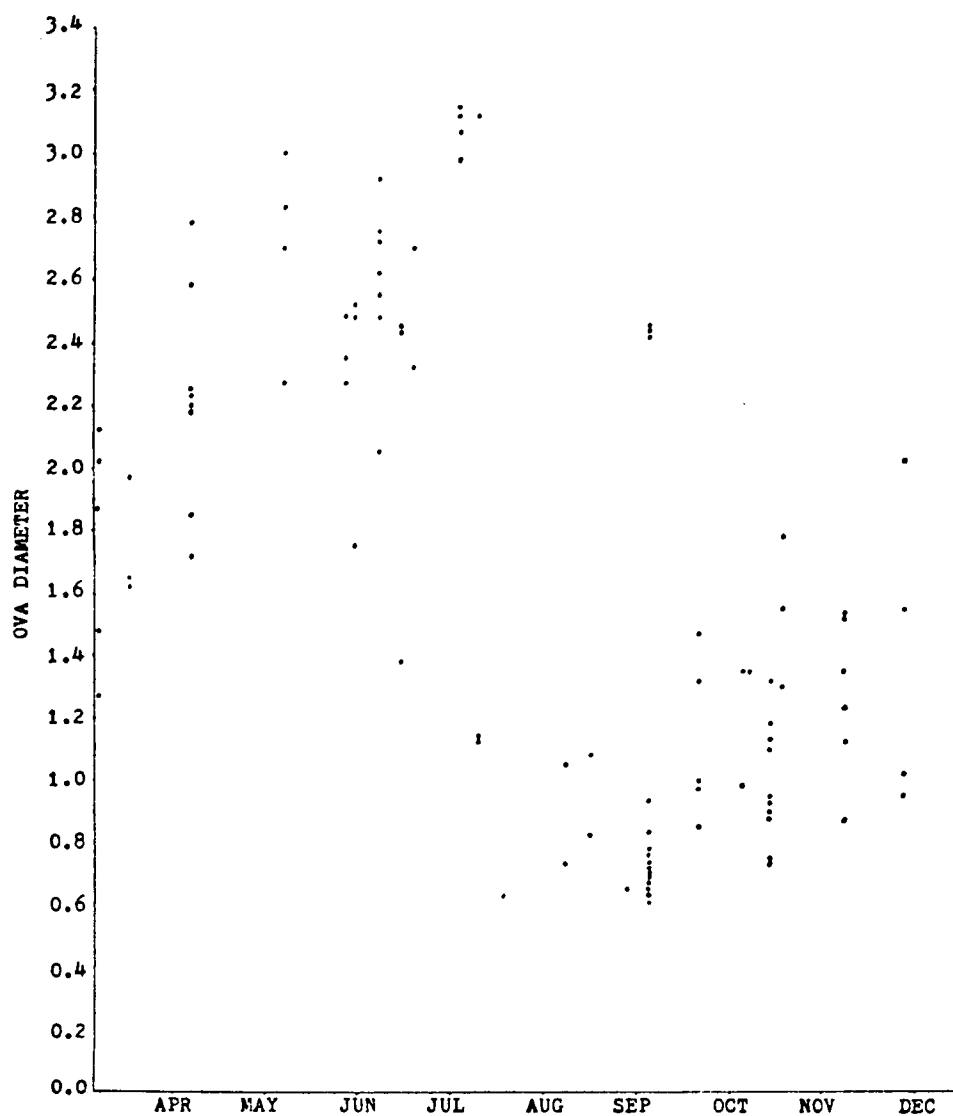


Figure 6. Maximum follicle diameter plotted against date of collection for mature *Desmognathus fuscus* females. No collections were made during January and February.

oviposition took place near the middle of June. The majority of fuscus probably oviposit from late June through early August.

Maximum egg sizes in santeetlah occur during the second week in June (Figure 7), approximately one month earlier than in fuscus. The smallest ovarian eggs in adult santeetlah were found from mid-July to early August; however, there is much greater variation in follicle sizes throughout the year in this species. The earliest date for santeetlah clutches in the field was 25 June. However, since several clutches collected at approximately the same time had embryos in early or intermediate stages of development, oviposition probably takes place from mid-May through early July.

Twenty-four of 25 fuscus clutches maintained in the laboratory hatched. Fifteen of these had either undifferentiated ova or were collected while the female was in the process of laying. The incubation period for these 15 clutches ranged from 34 to 60 days ($\bar{x} = 47.6$). Two clutches collected while oviposition was in progress required 56 and 60 days to hatch. Hatching dates in the laboratory for this species ranged from 23 August to 23 September.

Only 13 of 28 santeetlah clutches hatched in the laboratory. In all cases in which hatching did not occur, the eggs were either eaten by the attending female or molded after they were deserted. Of the 13 that hatched, only two were composed of ova in a relatively undifferentiated state at the time of collection. One of these was collected while the female was laying, and in this case 50 days were required for hatching. The other clutch was collected on 25 June and

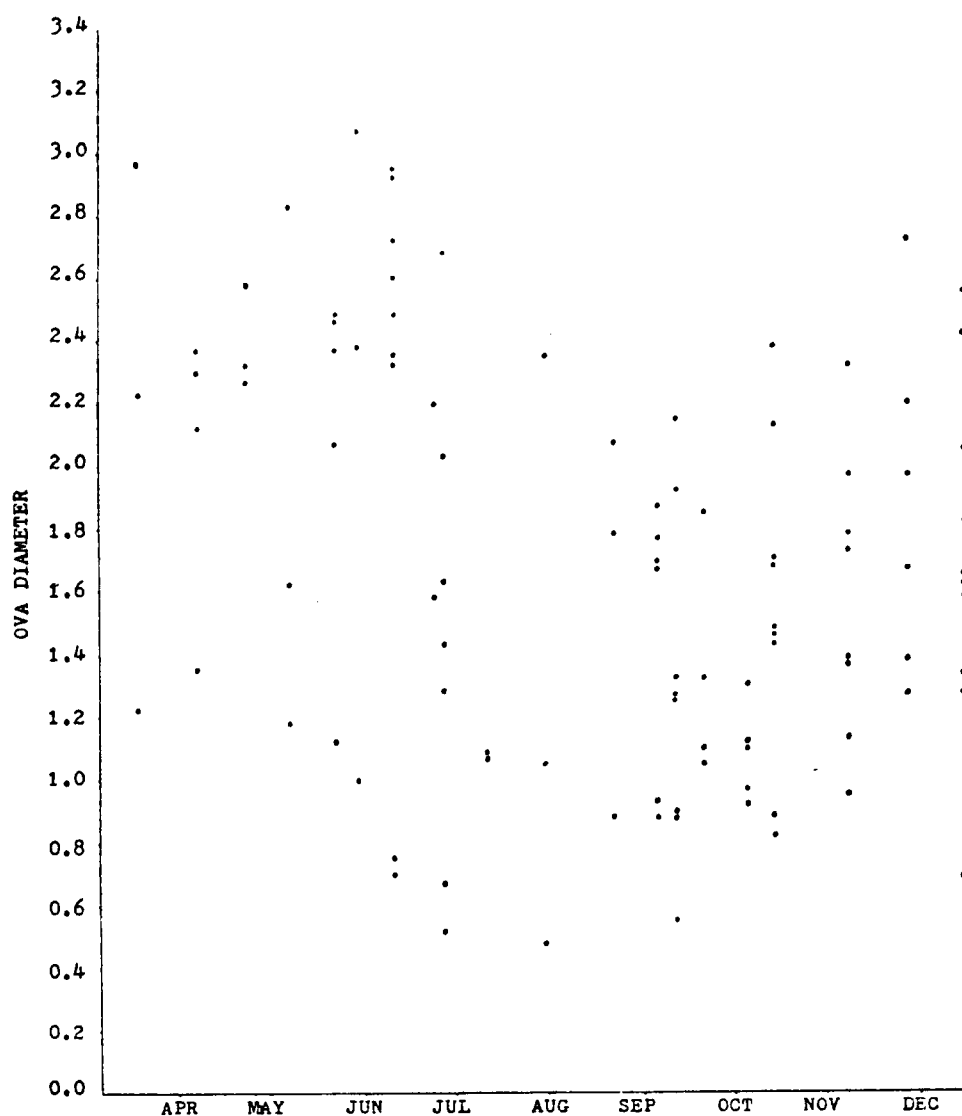


Figure 7. Maximum follicle diameter plotted against date of collection for mature Desmognathus santeetlah females. No collections were made during January, February and March.

hatched on 11 August, a period of 48 days. Hatching dates in the laboratory for this species ranged from 5 July to 24 August.

Most of the 25 fuscus clutches found were under rocks along the banks of streams or seepages (Table 42). Most of these were in contact with the soil. The mean distance of the fuscus nest sites from open water was 64.8 cm (range 30-99 cm). The majority of santeetlah clutches were beneath mosses growing on rocks, on logs, or on the soil surface (Table 42). Nest sites in this species ranged from 16 to 83 cm from the nearest open water ($\bar{x}$ = 56.2 cm). In many cases, these measurements were vertical distances from water, since this species commonly nests on logs over seepage areas. In both species, nesting chambers were oval or suboval hollows apparently made by the movements of the female. The eggs were grouped in grape-like clusters suspended from the ceiling of the nesting chamber. All three females collected while in the process of laying were lying on their dorsal surfaces. None of the clutches of either species were found in direct contact with water, as has been previously reported for fuscus (Organ, 1961).

Discussion

Males of these two species have many similarities in their reproductive biology. In both adults and juveniles, the reproductive characteristics that were examined, after transformation to principal components scores, showed a higher correlation to body size than to time of collection. Among juvenile males, the relationship between PC scores and body size reflects the gradual maturation of the

Table 42. Cover and substrates for 25 Desmognathus fuscus and 28 D. santeetlah clutches. Percentages in parentheses.

	D. fuscus	D. santeetlah
Cover		
Burrow	2(8)	1(4)
Rock	16(64)	1(4)
Moss	7(28)	24(85)
Log	0(0)	2(7)
Substrate		
Soil	20(80)	5(18)
Rock	2(8)	5(18)
Log	3(12)	18(64)

reproductive characters as size, and presumably age, increases. In adult males, the proportions of the variances of PC scores explained by body size were lower because the majority of characters examined (particularly vasa shape, vasa pigmentation, mental gland condition, and sperm content of the vasa) apparently change little once maturity is attained. The positive correlation between body size and PC scores results from including testis lobe number and vasa diameter in the analyses. The former is known to increase with body size in Desmognathus, and absolute value of the latter probably does so as well.

Juterbock (1978) stated that adult male Desmognathus do not show seasonal variation in vasa pigmentation or vasa shape, in apparent contrast to seasonal variation in those characters in many species of Plethodon. The results of this study confirm Juterbock's results, but also show that there is little seasonal variation in the amount of sperm in the vasa. In both species, at least 75 percent of the adult males collected in each month had vasa packed with sperm (Table 33, p. 68). This is also in apparent contrast to what occurs in other species of North American plethodontids (e.g., Bruce, 1978; Nagel, 1977, 1979), although aseasonal sperm production has been observed in tropical plethodontids (Houck, 1977). Other studies of male Desmognathus reproductive biology have either implied (Juterbock, 1978) or found (Huheey and Brandon, 1973) evidence for seasonal variability in sperm production. In the latter study, D. ochrophaeus males had packed vasa from September through June, and the authors concluded that spermatophore production could have occurred at any

time during that period. In this study, there was a slight reduction in the percentages of males with sperm packed vasa and smaller vasa diameters in mid to late summer. However, if the presence of abundant sperm in the vasa means that spermatophore production is possible, then most of the male fuscus and santeetlah in the study area could have been producing spermatophores in all months from which samples were taken. Alternatively, the vasa sperm observed during the summer months might have been residual and in the process of being resorbed. However, no overt structural differences between sperm during the summer and at other times of the year were observed.

Adult females of both species exhibit relatively pronounced seasonal variability in the condition of their reproductive organs, but this variation appears to be more regular in fuscus. An example of this is illustrated by the plots of mean ovum diameters versus date of collection. In fuscus (Figure 6, p. 85) most females have average maximum ova diameters exceeding 2.0 mm from late March through early July. From late July through December, maximum diameters are generally less than 1.6 mm. Variation of this sort has been interpreted as indicative of an annual oogenic cycle in other populations of fuscus (Spight, 1967; Danstedt, 1975; Hall, 1977). Desmognathus santeetlah shows a bimodal distribution of ova sizes through time (Figure 7, p. 87). In spring and early summer, one group of females had ova diameters greater than 2.0 mm, the other was composed of females with ova diameters of less than 1.8 mm. During late summer and autumn, the two groups were less distinct, but approximately half had ova greater and half smaller than 1.6 mm in diameter. This

pattern has been interpreted in a variety of ways. Organ (1961) found a bimodal distribution of ova sizes in five species of Virginia Desmognathus and felt that it represented a biennial oviposition cycle. Winter oviposition occurs in some populations of D. ochrophaeus (Tilley and Tinkle, 1968; Tilley, 1973b), which results in spent females in the spring and gravid females in the fall. Mark-recapture studies of D. ochrophaeus (Tilley, 1977) indicated that an annual oogenic cycle was predominant in the populations studied, but that much individual variation was present, resulting in gravid females in late summer and spent females in the spring. Tilley (1977) also found that some females carrying enlarging follicles apparently failed to oviposit during a given summer, and concluded that few females maintained an annual cycle throughout their lives. Without mark-recapture data, no definitive statements concerning oogenic cycles of the two species in this study are possible. However, if Tilley's (1977) results are representative of oogenic cycles in the genus, then both species may have primarily annual cycles. The bimodal distribution of ova diameters in santeetlah may result from both winter and summer oviposition; however, no brooding females nor recently hatched larvae have been observed in the study area during the winter. If winter oviposition does not occur, then the bimodal distribution might result from greater individual variation in oviposition schedules in this species.

Both species produce on the average about the same number of eggs per clutch. However, santeetlah is smaller than fuscus, and when corrected for snout-vent length, clutch size is significantly larger

in the former. Therefore, santeetlah has a greater reproductive effort than fuscus. The data on clutch volume-body volume relationship also support this hypothesis, since santeetlah has a significantly higher clutch volume-body volume ratio. Other studies of relative clutch sizes in Desmognathus have found differences intraspecifically (Danstedt, 1975) or interspecifically (Tilley, 1968; Hall, 1977). In each case, greater fecundity was hypothesized to be associated with higher mortality relative to other populations or species.

Differences in time of oviposition may be a result of differences in the elevational distribution of the two species. Tilley (1973b) estimated that high elevation populations of D. ochrophaeus on Mt. Mitchell, North Carolina, oviposited an average of 18 or more days earlier than did low elevation populations. This was thought to result from a faster yolking rate at higher elevations.

The length of the incubation period for both species ranges from 45 to 60 days in the laboratory. Estimates of the length of the incubation period for field clutches of fuscus range up to as many as 89 days (Dennis, 1962), although Danstedt (1975) considered this figure to be excessive. Noble and Evans (1932) reported that fuscus clutches kept in the laboratory hatched in 39 to 52 days, but stated that laboratory temperatures were probably higher than those of natural nests. If temperatures influence the length of time required for hatching, then the incubation period for both species is probably longer than that given above, since laboratory temperatures were probably higher and less variable than nest temperatures in the field.

Laboratory temperatures may also have played a part in the lower hatching success of the santeetlah clutches. This species occurs at higher elevations where temperatures are generally lower, so the higher laboratory temperatures may have either affected normal development of the larvae or the brooding behavior of the attending females.

The major difference in the nest sites of these two species was in the type of cover under which brooding females were found. Most santeetlah clutches were beneath moss, while most fuscus clutches were under rocks along stream banks. This difference may be due to the relatively greater availability of mosses in santeetlah habitats, since there was a higher percentage of organic matter (primarily mosses) in those habitats (Table 18, p. 36). However, most fuscus streams also had moss-covered logs and rocks along their banks, yet few fuscus used those mats as nesting sites (Table 42, p. 89). The relative sizes of the females may also play a part in determining the nesting sites selected by each species. Most of the moss mats in the study area are from 1-3 cm in thickness, so the smaller santeetlah females might find it easier to establish brooding chambers under growths of moss. Alternatively, greater precipitation in the higher elevation santeetlah habitats may result in higher and more constant moisture conditions beneath mosses, which would make them more suitable as nesting sites relative to mosses at lower elevations.

Both species appear to have males that are capable of producing spermatophores aseasonally and females with essentially seasonal reproductive cycles. Houck (1977) hypothesized that, in Bolitoglossa rostrata, this pattern results from males being presented with at

least some opportunities to inseminate females at all seasons. As a result, males must undergo continual spermatogenesis in order to avoid missing such opportunities. It does not seem likely that male Desmognathus undergo continual spermatogenesis, since the lobed testis of these salamanders arises as a result of apparently discontinuous spermatogenic activity (Humphrey, 1922). The question, then, is whether sperm packed vasa are an indication of potential reproductive activity. Most workers have assumed that they are (e.g., Huheey and Brandon, 1973). If so, then male Desmognathus may be able to store viable sperm in the vasa in the absence of active spermatogenesis. This would require some mechanism that would allow for nourishment of the stored sperm. Female fuscus have spermatheca in which sperm appear to obtain nourishment from the epithelial lining (Marynick, 1971), so there might be a similar mechanism involving the lining of the vasa. Less than packed vasa during the summer may indicate the period during which no spermatogenic activity is taking place, and the males with such vasa are those in which vasa evacuation has taken place without replacement by testicular sperm; i.e., those few males without packed vasa may have previously engaged in spermatophore production.

V. POPULATION STRUCTURE, DENSITY, AND PREDATION

This section is concerned with aspects of life history and demography, including population structure, densities, and indirect analyses of predation pressure. These may provide information on the adaptive strategies of the two species in the Unicoi Mountains. Most studies of the life history and demography of plethodontid salamanders have used the vertical approach (Tilley, 1977). This involves examining snout-vent length distributions of chronological samples for distinct size classes which presumably represent age classes. The vertical approach is used because there is no accurate method of ageing salamanders. Previous investigators of desmognathine life histories have attempted to construct life tables for males based upon testes lobe counts. The number of lobes was thought to be an index of age, with one complete lobe added to each testis approximately every two years (Humphreys, 1922). However, Tilley (1977) found that testes lobe number and age were only roughly correlated in Desmognathus ochrophaeus, and that lobe number could only indicate whether a male was either relatively young or relatively old. Therefore, the information to be gained from the vertical approach is necessarily limited to estimates of age at transformation, age at sexual maturity, and rough comparisons of male age structure based on testes lobe number.

Methods

Specimens upon which these analyses are based are those used in the sections on morphology and reproduction. Collections were

grouped into seasonal samples representing spring (March through May), early summer (June and July), late summer (August and September), and autumn (October through December). These samples provided information on sex ratios, age and season of transformation, and age and season of sexual maturity. Adult male testes lobe distributions were also compared.

Density estimates were obtained from mark-recapture studies conducted over three day periods in 1980. The dates of these studies were 20-22 September for santeetlah and 26-28 September for fuscus. Three localities were chosen for each species. At each locality, two plots were randomly selected and sampled. Plots were 5 m in length and at least 5 m distant from one another. The areas of the sample plots were estimated by measuring the width of the streambed or seepage area at each end of the plot, adding 1 m of bank on each side, and calculating the area of the resulting four-sided figure. Each plot, including the 2 m of bank, was searched for 30 min. In many cases, the entire plot was covered in less than 30 min., so parts of some plots were searched twice in one sample period. Searches were made during the daylight hours on each of the three days.

Animals were uniquely marked by clipping one toe on at least two feet. For juveniles too small to toe-clip, the tail was notched in such a way so that it could be distinguished from a natural injury.

Two methods of estimating densities were used. Both were calculated as described in Tanner (1978). The first method (Lincoln-Peterson method, or single mark-recapture estimate) used

data from the initial marking period and the first recapture period. Population size (N) was estimated as equal to $M(C+1)/(R+1)$, with an expected variance of $M^2(C+1)(C-R)/(R+1)^2(R+2)$, where M is the number of marked individuals in the population, C is the total number in the sample from the first recapture period, and R is the number of marked animals in the sample C . This procedure assumes that marking does not affect the probability of recapture, that marks were not lost, and that no death or emigration occurred between the time of release and recapture.

The second method (Jolly-Seber method, or multiple mark-recapture estimate) used data from the initial marking period and both recapture periods. Population size (N_2) during the first recapture period was estimated as $M_2 C_2 / R_{12}$, where M_2 is the estimated size of the marked population during the first recapture period, calculated as $(F_2 R_{13} / R_{23}) + R_{12}$, where C_2 is the total number of individuals captured during the first recapture period, F_2 is the number released at the end of that period, R_{12} is the number of marked individuals recaptured in the first recapture period, R_{13} is the number of marked individuals recaptured only in the second recapture period, and R_{23} is the number of individuals recaptured in both recapture periods. The expected variance of this estimate is equal to $N_2(N_2 - C_2) [(M_2 - R_{12} + F_2 / M_2)(1/R_{23} - 1/F_2) + 1/R_{12} - 1/C_2]$. In some cases M_2 was greater than the known number of marked individuals in the population at that time, which invalidated the estimate. The multiple mark-recapture procedure has the same assumptions as the

the single mark-recapture, except that the population is not assumed to be closed. Both methods were used because they allow for estimates during the first recapture period. The assumptions of both procedures were not considered a problem, since the recapture periods were approximately 24 hours apart and little death or emigration would be expected to occur, and because the method of marking made it difficult to overlook marked individuals.

Potential predator and/or competitor species found in the sample plots were also marked and their densities estimated. The other species included both salamanders and crayfish. The salamanders were marked as described above; the crayfish by cutting small notches in at least two segments of the tail section. Population estimates for all of the other species combined were also calculated.

Tail break frequencies have been used as an index to predation for both salamanders and lizards. One of the problems with determining the frequency of tail breaks in salamanders has been the difficulty in distinguishing a completely regenerated tail from one that has never been broken. Shaffer (1978) proposed a method of surmounting this problem based on linear regression. Tail length is regressed on snout-vent length using animals with tails that have presumably never been broken. This results in an equation from which a predicted tail length can be calculated for any snout-vent length. Specimens are then evaluated as to whether actual tail lengths are greater than a statistically predetermined prediction limit. The prediction limit

is defined as $Y_k - t_\alpha (S_{y.x}^2 (1 + 1/N + (X_k - \bar{X})^2 / (X_i - \bar{X})^2))^{1/2}$,

where Y_k is the predicted tail length of the individual in question, t_α is the percentile of Student's t-distribution with N-2 degrees of freedom, $S_{y.x}^2$ is the variance of tail lengths of the individuals in the regression analysis, X_i is the snout-vent length of each individual in the regression analysis, N is the sample size, X_k is the snout-vent length of the individual in question, and $\bar{X}$ is the mean snout-vent length of the individuals in the regression analysis. Shaffer felt that $\alpha=.90$ yielded a reasonable estimate for his purposes.

Raw and predicted tail break frequencies were compiled for each sex and maturity class. The predicted frequencies were calculated using regression equations for each individual sex and maturity class, rather than species-wide equations as used by Shaffer (1978). Lower prediction limits were calculated using $\alpha=.90$.

Results

Age at Transformation and Sexual Maturity

Snout-vent length distributions for the four seasonal periods are shown in Figure 8 for santeetlah and in Figure 9 for fuscus. Both species oviposit during the summer, and hatching occurs at least two months later, which means that most santeetlah hatch in mid-August and most fuscus hatch in mid-September. The aquatic larvae at hatching range in size from about 8-9 mm SVL in santeetlah and 9-12 mm SVL in fuscus (Tilley, 1981). The smallest transformed juveniles were collected during the summer and were 9 and 12 mm

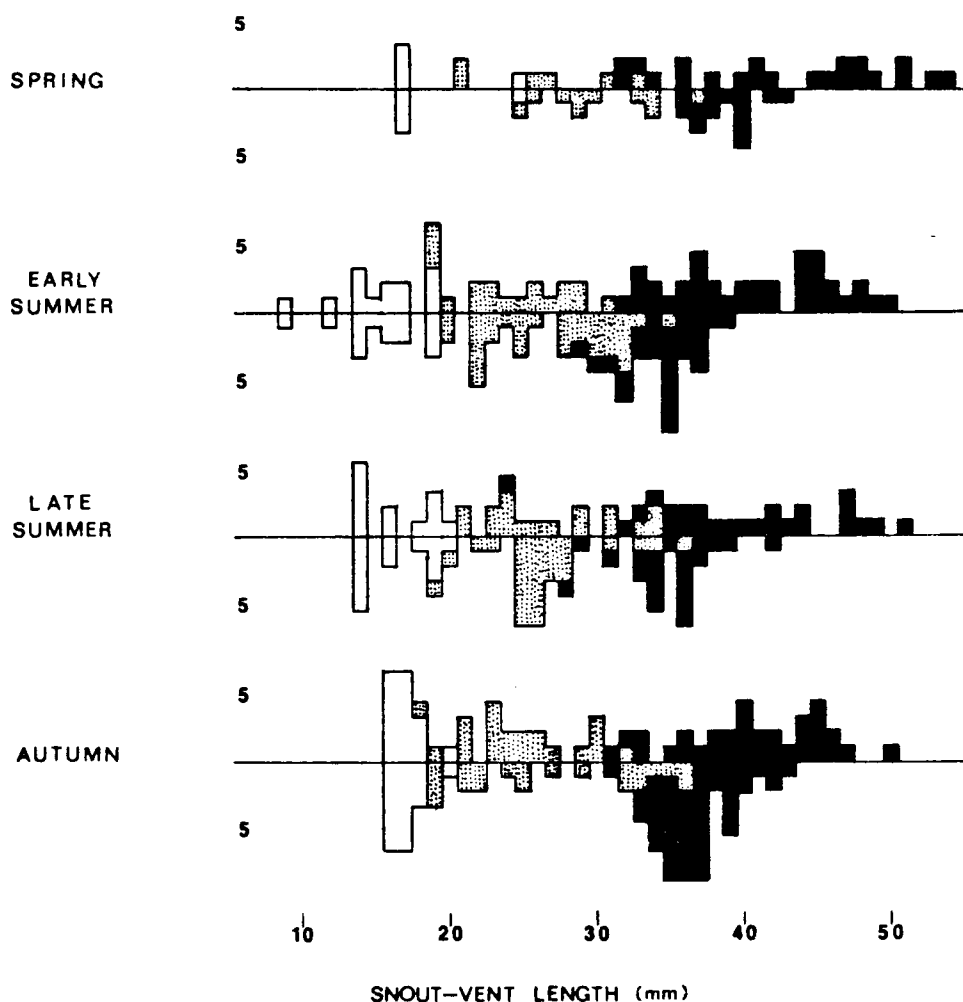


Figure 8. Snout-vent length distributions of *Desmognathus santeetlah* from the four seasonal periods. Males are above the horizontal line, females below it, and unsexed juveniles are plotted on both sides of the line. Open squares represent unsexed juveniles, stippled squares sexed juveniles, and solid squares represent adults.

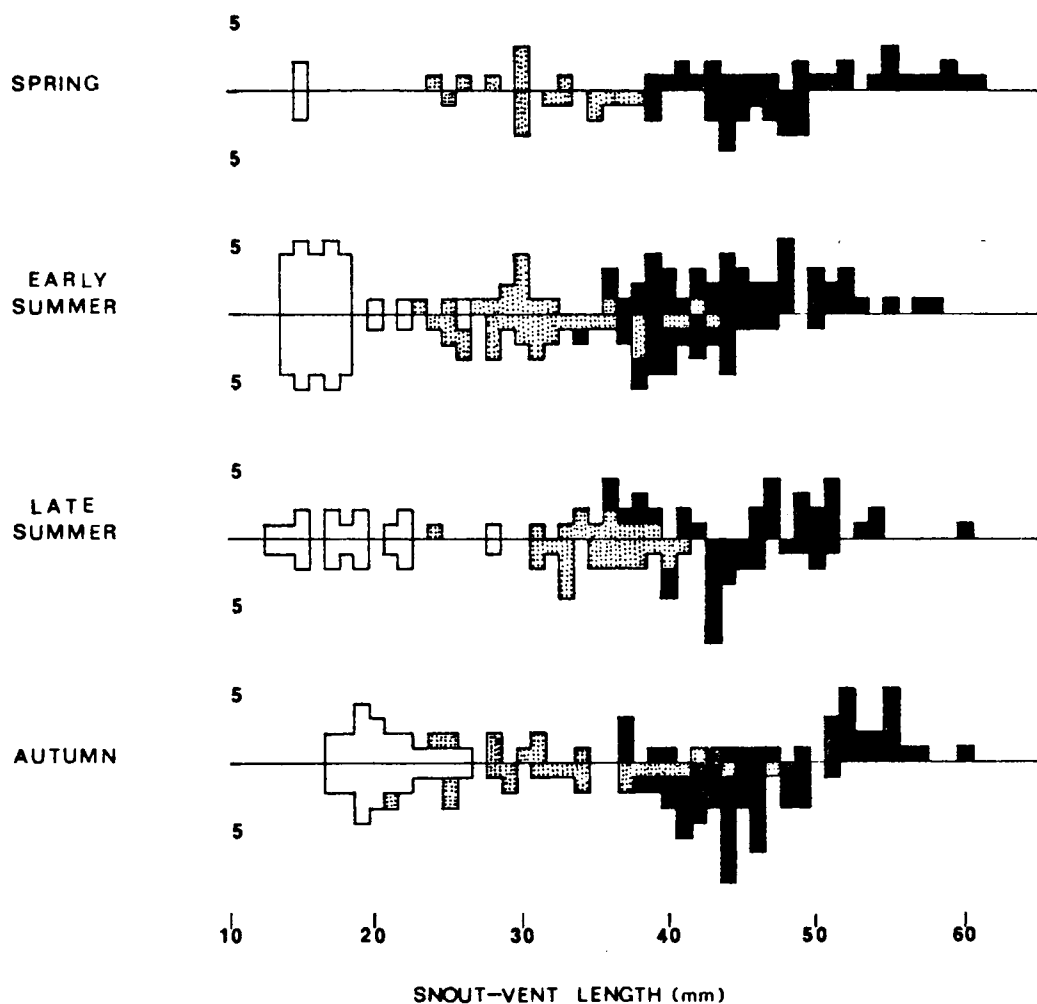


Figure 9. Snout-vent length distributions of *Desmognathus fuscus* from the four seasonal periods. Males are above the horizontal line, females below it, and unsexed juveniles are plotted on both sides of the line. Open squares represent unsexed juveniles, stippled squares sexed juveniles, and solid squares represent adults.

SVL in santeetlah and fuscus, respectively (Figures 8 and 9). This suggests that the larval period in both species is slightly less than a year in length, since recently transformed individuals were only a few mm larger than larvae. In the autumn samples, some juvenile santeetlah less than 20 mm SVL were sexable while others were not. The same situation occurred in fuscus juveniles less than 25 mm SVL. This suggests that juveniles become sexable in their first autumn and winter after transformation, when they are slightly over one year of age.

The SVL distributions of juvenile males were unimodal in both species. Juvenile females, however, had bimodal distributions of SVLs during at least part of the year, which is usually interpreted as indicative of the presence of at least two age classes in the sample (eg. Danstedt, 1975). A bimodal distribution is present in the early summer samples of juvenile females of both species. In santeetlah, one peak occurs at approximately 20 mm SVL and the other at approximately 30 mm SVL; in fuscus, the two peaks are at approximately 30 and 38 mm SVL. Among juvenile males of both species, there is one size class present during the spring, which represents individuals that transformed and became sexable the preceding autumn. These individuals are over one year of age but less than two years of age. By late summer these juveniles have attained considerable size and average approximately 30 mm SVL in santeetlah and 35 mm SVL in fuscus (Figures 8 and 9). The absence of these large juveniles in the autumn samples indicates that they probably attained sexual

maturity between those two times, when they were approaching their second birthday. In juvenile females, the two size classes that are present in early summer indicate that there are at least two age classes represented. The larger of these will transform between late summer and autumn, while the smaller will remain juvenile for at least another year. Therefore, juvenile males of both species become sexually mature during late summer when they are approximately two years of age while females become sexually mature approximately one year later at three years of age.

Testes Lobe Distributions

The testes lobe distributions of adult males of both species are shown in Table 43. A test for homogeneity of the distributions showed that they did not differ significantly ($G = .06$, $p < .50$). These results indicate that relative age distributions of males are approximately the same in the two species, assuming that the lobes develop in the same manner in both.

Sex Ratios

Sex ratios of santeetlah regardless of maturity class were not different from 1:1 ($\chi^2 = .273$, $p < .50$). The same was true for fuscus ($\chi^2 = .103$, $p < .50$). However, when maturity class was considered, juvenile females significantly outnumbered juvenile males in both species (santeetlah, $\chi^2 = 4.195$, $p < .05$; fuscus, $\chi^2 = 9.96$, $p < .01$). This resulted in sex ratios of 1.4:1.0 (female:male) for santeetlah and 1.8:1.0 for fuscus, which is a result of later ages at maturity for females. Among adults of both species, sex ratios were not

Table 43. Testes lobe distributions for adult males of Desmognathus fuscus and D. santeetlah. Percentages are in parentheses.

Lobe number	D. santeetlah	D. fuscus
1.0	50(43.1)	57(43.5)
1.5	34(29.3)	38(29.0)
2.0	22(19.0)	23(17.6)
2.5	7(6.0)	9(6.9)
3.0+	3(2.6)	4(3.0)

different from 1:1 (santeetlah, $\chi^2 = 1.037$, $p < .10$; fuscus, $\chi^2 = 3.35$, $.05 < p < .10$).

Mark-recapture Estimates

Density estimates for fuscus are shown in Table 44. Mean densities were $1.26/\text{m}^2$ (Lincoln-Peterson) and $1.32/\text{m}^2$ (Jolly-Seber). Density estimates for individual species found in fuscus habitats and for all species considered together (excluding fuscus) are also shown in Table 44. Only those species for which there were enough recaptures to provide an estimate are shown. Mean density estimates for all species combined were $1.43/\text{m}^2$ (Lincoln-Peterson) and $1.72/\text{m}^2$ (Jolly-Seber). The total number of individuals captured in the fuscus plots, population estimates and variance estimates are shown in Table 45.

Density estimates for santeetlah are shown in Table 46. Mean densities for this species were $3.27/\text{m}^2$ (Lincoln-Peterson) and $2.76/\text{m}^2$ (Jolly-Seber). Density estimates for other species found in santeetlah

Table 44. Density estimates ($\hat{N}/m^2$) for *Desmognathus fuscus*, for other species found in *D. fuscus* habitats, and for all species excluding *D. fuscus* in the six mark-recapture plots. L-P denotes Lincoln-Peterson estimates; J-S denotes Jolly-Seber estimates. * indicates that an estimate was not possible. Localities were: Number 1-Hell Hole Branch at Forest Service Road 35, elevation 293 m; Number 2-unnamed tributary of Citico Creek at Forest Service Road 35, elevation 299 m; Number 3-Gold Cabin Branch at Forest Service Road 26, elevation 488 m.

	Area(m^2)	D. fuscus		D. monticola		Crayfish		All	
		L-P	J-S	L-P	J-S	L-P	J-S	L-P	J-S
Locality 1									
Plot A	16.3	0.74	*	0.18	0.12	*		0.61	0.43
Plot B	24.1	0.55	0.37	0.23	0.25	0.62	*	0.89	0.42
Locality 2									
Plot A	15.3	1.83	1.95	0.29	*	0.13	*	0.44	*
Plot B	16.6	3.79	3.65	*	*	1.05	*	1.27	*
Locality 3									
Plot A	18.5	0.43	0.38	0.08	0.11	*		0.02	0.22
Plot B	16.5	0.20	0.24	*	*	1.94	0.91	4.04	5.82

Table 45. Population estimates ($\hat{N}$), variance estimates (s^2), and total number of captures for each species in the Desmognathus fuscus mark-recapture plots. L-P denotes Lincoln-Peterson estimates; J-S denotes Jolly Seber estimates. * indicates that an estimate was not possible. Localities as in Table 44.

Species	Locality	Plot	N		s^2		Number of Captures
			L-P	J-S	L-P	J-S	
<u>D. fuscus</u>	1	A	12.0	*	18.0	*	17
	1	B	13.3	9.0	4.9	0.0	28
	2	A	28.0	29.9	56.0	139.1	41
	2	B	62.9	60.6	336.7	415.3	68
	3	A	8.0	7.0	10.0	0.0	13
	3	B	3.3	4.0	1.1	0.0	9
<u>D. monticola</u>	1	A	3.0	2.0	1.0	0.0	8
	1	B	5.6	6.0	1.5	0.0	12
	2	A	4.5	*	2.3	*	7
	2	B	*	*	*	*	4
	3	A	1.5	2.0	0.3	0.0	4
	3	B	32.0	15.0	277.3	0.0	12
Crayfish	1	A	*	*	*	*	7
	1	B	15.0	*	22.5	*	13
	2	A	2.0	*	0.0	*	3
	2	B	17.5	*	72.9	*	12
	3	A	*	*	*	*	1
	3	B	32.0	15.0	277.3	0.0	25
<u>D. quadramaculatus</u>	1	A	*	*	*	*	3
	1	B	*	*	*	*	4
	2	B	*	*	*	*	1
	3	B	*	*	*	*	8
<u>D. aeneus</u>	3	B	*	*	*	*	2
All	1	A	10.0	7.0	10.0	0.0	18
	1	B	20.4	10.0	18.9	0.0	29
	2	A	6.7	*	2.8	*	10
	2	B	21.0	*	10.50	*	17
	3	A	3.3	4.0	1.1	0.0	7
	3	B	66.7	96.0	977.8	0.0	45

Table 46. Density estimates ($\hat{N}/m^2$) for *Desmognathus santeetlah*, for other species found in *D. santeetlah* habitats, and for all species excluding *D. santeetlah* in the six mark-recapture plots. L-P denotes Lincoln-Peterson estimates; J-S denotes Jolly Seber estimates. * denotes that an estimate was not possible. Localities were: Number 1-seepage at Sugar Cover Branch and Forest Service Road 217, elevation 841 m; Number 2-seepage at Meadow Branch and Forest Service Road 217, elevation 1164 m; Number 3-seepage below Mud Gap at Forest Service Road 217, elevation 1286 m.

	Area(m^2)	<i>D. santeetlah</i>		<i>D. ochrophaeus</i>		<i>D. quadramaculatus</i>		All	
		L-P	J-S	L-P	J-S	L-P	J-S	L-P	J-S
Locality 1									
Plot A	13.6	0.22	0.15	*	*	*	*	4.04	0.74
Plot B	18.2	0.55	*	0.17	0.11	*	*	1.71	1.44
Locality 2									
Plot A	17.5	8.69	7.33	0.17	0.11	0.06	0.06	0.23	0.17
Plot B	15.0	5.70	2.81	0.13	0.07	*	*	1.00	*
Locality 3									
Plot A	16.1	3.11	3.07	0.33	0.44	*	*	0.56	0.50
Plot B	19.5	1.37	0.46	0.45	0.48	0.27	0.31	0.81	*

habitats are also shown in Table 46, along with densities of all other species combined. Mean estimates for the latter were $1.39/\text{m}^2$ (Lincoln-Peterson) and $0.66/\text{m}^2$ (Jolly-Seber). Total individuals captured, population estimates, and variance estimates for these groups are shown in Table 47.

Lincoln-Peterson density estimates for both fuscus and santeetlah were compared with those of all other species combined using Spearman rank correlation. There was a significant inverse correlation between santeetlah densities and the combined densities of other species in the sample plots ($r = -.8286$, $p = .029$), but none between fuscus densities and the densities of other species ($r = -.0857$, $p = .46$). There were no significant correlations between either fuscus or santeetlah densities and those of any other individual species.

Tail Break Frequencies

Raw and predicted tail break frequencies for both species are shown in Table 48. Mean frequencies were higher in fuscus for almost all age and maturity class combinations. In general, predicted frequencies were higher for adults than juveniles; however, juvenile fuscus had tail break frequencies that were almost as high as those of adults.

Discussion

Snout-vent length and testes lobe distributions, as well as ages at transformation and sexual maturity, are similar for the two

Table 47. Population estimates ($\hat{N}$), variance estimates (s^2), and total number of captures for each species in the Desmognathus santeetlah mark-recapture plots. L-P denotes Lincoln-Peterson estimates; J-S denotes Jolly-Seber estimates. * indicates that an estimate was not possible. Localities as in Table 46.

Species	Locality	Plot	N		s^2		Number of Captures
			L-P	J-S	L-P	J-S	
<u>D. santeetlah</u>	1	A	3.0	2.0	0.0	0.0	6
	1	B	10.0	*	5.0	*	18
	2	A	152.0	128.3	2166.0	4622.8	89
	2	B	85.4	42.2	1269.2	357.3	68
	3	A	50.0	49.5	131.3	3379.6	31
	3	B	26.7	9.0	124.8	0.0	27
<u>Pseudotriton ruber</u>	1	A	1.0	1.0	0.0	0.0	3
	1	B	*	*	*	*	1
<u>Gyrinophilus porphyriticus</u>	1	A	9.0	5.0	18.0	0.0	9
	1	B	*	*	*	*	11
<u>D. monticola</u>	1	A	*	*	*	*	9
	1	B	10.0	4.0	10.0	0.0	13
<u>D. ochrophaeus</u>	1	A	*	*	*	*	7
	1	B	3.0	2.0	0.0	0.0	11
	2	A	3.0	2.0	0.0	0.0	6
	2	B	2.0	1.0	0.0	0.0	6
	3	A	5.3	7.0	4.4	0.0	14
	3	B	8.8	9.3	3.1	46.6	16
<u>D. quadramaculatus</u>	1	A	*	*	*	*	3
	2	A	1.0	1.0	0.0	0.0	5
	2	B	*	*	*	*	7
	3	A	*	*	*	*	2
	3	B	5.3	6.0	2.4	0.0	12
All	1	A	55.0	10.0	55.0	0.0	35
	1	B	31.2	26.1	94.6	343.5	32
	2	A	4.0	3.0	0.0	13.5	11
	2	B	15.0	*	11.3	*	13
	3	A	9.0	8.0	13.5	0.0	16
	3	B	15.7	*	11.2	*	28

Table 48. Raw and predicted tail break frequencies for Desmognathus fuscus and D. santeetlah. Numbers listed are percentages of each group with broken tails.

	<u>D. santeetlah</u>		<u>D. fuscus</u>	
	Raw	Predicted	Raw	Predicted
Adult male	25.00	43.10	22.14	46.56
Adult female	15.84	34.65	16.50	39.80
Juvenile male	9.68	16.13	24.39	36.58
Juvenile female	13.79	28.74	28.00	46.67
Unsexed juvenile	20.45	20.45	25.00	21.67
Overall $\bar{X}$	16.95	28.65	23.21	38.26
$\bar{X}$ Adult	20.42	38.88	19.32	43.18
$\bar{X}$ Juvenile	14.64	21.77	25.80	34.97

species. The implication of this is that population structures are therefore similar. Age at maturity has been estimated for fuscus as between two and three years for males and a year later for females (Danstedt, 1975; Hall, 1977; Organ, 1961), so the estimates given here are probably realistic.

The density estimates are also similar to those reported in other studies of fuscus (Danstedt, 1975; Hall, 1977; Orser and Shure, 1975; Spight, 1967). However, they should be considered as minimal estimates since they were from diurnal samples taken in late summer. Orser and Shure (1975) found that nocturnal estimates of population sizes were consistently higher than diurnal ones, and that the highest estimates occurred in the spring. The higher estimates for santeetlah may have resulted either from seasonal

bias or from the nature of the habitats occupied by each species. Density estimates in late September may be underestimates for fuscus, since it is possible that some females were still brooding when the population estimates were taken. Desmognathus santeetlah, which nests a month earlier in the study area, would not be expected to show such a bias. Secondly, fuscus in the study area are found primarily along the banks of streams, while santeetlah are found in seepage areas. The latter had more available habitat per square meter of sample plot than did the stream beds. Therefore, higher density estimates for santeetlah may simply indicate greater habitat availability per square meter of seepage area than in stream beds. This is suggested by the fact that the highest fuscus density estimates were from a stream with extensive gravel bars and little open water. The significant inverse correlation between santeetlah densities and those of other species may indicate that it is more sensitive to interspecific interactions than fuscus, which showed no such correlation. The nature of these interactions, if they occur, is unclear, since no individual species showed any correlation with santeetlah densities.

Tail break frequencies have been used in several studies of Desmognathus biology as an index to predation pressure. Hall (1977) found that 17.6 percent of the population he examined had broken tails, with the highest frequency among adult males. Danstedt (1975) found that populations with higher estimated survival rates also had lower tail break frequencies. In this study, fuscus had higher tail break frequencies, which might mean that it experiences relatively

greater predation pressure. However, Schoener (1979) has questioned the use of tail break frequencies as an indication of the intensity of predation, pointing out that a population composed entirely of individuals with complete tails could result from predators that were 100 percent efficient, capturing all individuals attacked, or from no predation at all. Therefore, lower tail break frequencies in santeetlah could mean that its predators are more efficient than those of fuscus.

VI. SUMMARY AND CONCLUSIONS

Desmognathus santeetlah and D. fuscus differ in morphology, color pattern, and in certain aspects of their reproductive biology. The latter is larger in overall size with a relatively longer tail and rear limbs. It also has a characteristically brighter and bolder pattern. Desmognathus fuscus appears to have a more seasonally defined reproductive period than santeetlah, and usually oviposits an average of approximately a month later. Both species produce approximately equal number of eggs per clutch, which, because of its smaller body size, indicates that santeetlah has a relatively greater reproductive output. Population structure, the larval period, and age at sexual maturity appear to be similar in the two species. There is some evidence that fuscus experiences a higher predation rate among the juvenile classes, while santeetlah population density is inversely correlated with the density of co-occurring species.

Based on electrophoretic and morphological evidence, santeetlah is more closely related to fuscus than to the other species in the genus (Tilley, 1981). Considering this and the present distribution of the two, it seems likely that santeetlah is derived directly from fuscus or from fusoid stock that became isolated in the high elevation seepage areas of the Southern Appalachians. Its subsequent divergence has in many ways mirrored the events that occurred during the evolution of the genus as a whole. In general, the trend within the genus has been toward increasing terrestriality and smaller body size (Dunn, 1926; Wake, 1966). The latter has necessarily resulted in a smaller absolute

number of ova produced per clutch, but this has been counterbalanced somewhat by a reduced larval period and increased survivorship of the terrestrial adults.

Given the apparent close relationship between the two species, what are the possible factors that led to the differences in morphology and reproduction enumerated above? As suggested earlier, many of the differences in morphology and color pattern seem to be adaptations to the different habitats occupied by each species. Despite the gross similarity in some characteristics of their habitats, santeetlah is primarily a species of seepages while fuscus reaches its greatest densities along small streams in the study area. Smaller body sizes, reduced rear limbs, and duller patterns would be advantageous to santeetlah in shallow seepages with abundant organic matter, while brighter patterns, larger body sizes and both longer legs and tails would be advantageous in the rocky substrates of the streambeds occupied by fuscus at lower elevations. The apparently high predation rates on juvenile fuscus, if in fact that is what high tail break frequencies indicate, might also mean that it is relatively risky to be small in the low elevation streambeds, implying selective pressures for large adult size.

Differences in some aspects of life history are also relatively pronounced. Desmognathus santeetlah has a relatively higher reproductive output than fuscus. One explanation for this is that females of santeetlah, as a result of their semiaquatic brooding and smaller size, experience relatively greater predation pressure, and as such, selection has favored greater reproductive efforts per season. This hypothesis

as been used to explain relatively greater fecundities in other populations of fuscus (Danstedt, 1975; Tilley, 1968). However, data on population structure and tail break frequencies run contrary to this, since the latter are smaller for all sex and age classes of santeetlah. Assuming that female population structure is similar to that of males, and that male population structure is at least partly reflected by testes lobe data, then population structures of the two species are similar. However, santeetlah had its lowest densities at a locality where both Pseudotriton ruber and Gyrinophilus porphyriticus were present, and both of these species are predators on small salamanders.

A second explanation for the greater reproductive effort of santeetlah involves the nature of the seepage habitats. These areas are less predictable than the streambeds occupied by fuscus. During dry periods, water flow in seepages often becomes greatly reduced. The probability of brooding a clutch to hatching would also be reduced relative to that in a more predictable habitat, so that selection would favor females that consistently produce larger clutches. In a dry year, both females with large clutches and those with smaller clutches would be expected to experience similar rates of brooding failure, while during wet years, the females with larger clutches would leave proportionately more offspring. The unpredictability of seepage areas might also explain other aspects of the reproductive biology of this species. Tilley (1981) noted the more larviform morphology of fuscus hatchlings, which suggests that this species

has a longer larval period than santeetlah. An unpredictable larval habitat would select for a shorter larval period in the latter. Although the appearance of recently transformed individuals in the field seems to indicate approximately equal larval periods, the recently transformed santeetlah might have been the result of fall or winter brooding. This is suggested by the relatively seasonal variation of ovarian ova sizes mentioned earlier. Aseasonal reproduction could also result from an unpredictable brooding environment. Most brooding in santeetlah takes place during mid-summer when rainfall is generally lower than during the rest of the year. Selection might then favor greater variability in the timing of reproduction relative to what occurs in fuscus. Considering these factors together, santeetlah appears to be a more r-selected species than fuscus. This would also account for the inverse correlation between densities, since one of the characteristics of such a species is poor competitive abilities (Pianka, 1970). This however, assumes that there is a cause-effect relationship between the densities of santeetlah and those of the other species that occur in the same habitats with it.

Finally, why are the ranges of the two species in the Unicois separate and nonadjacent; i.e., why aren't habitats at intermediate elevations, which are similar in many respects to those at higher or lower elevations, occupied by one or the other of the two species? Secondly, why is santeetlah a habitat specialist while fuscus is more generalist in nature, occupying both seepages and streambanks at lower elevations? The answer to the first question may involve interactions

between one or both of these species and other Desmognathus in the Unicoi Mountains. At low elevations, fuscus occurs with both D. monticola and to a lesser degree with D. quadramaculatus. However, neither of the latter two is very abundant at those elevations. Desmognathus santeetlah occurs with D. ochrophaeus and D. quadramaculatus in seepage areas, but the latter is much more common along streambanks. The only locality where santeetlah and D. monticola were found together was at 841 m elevation, the lowest elevation at which santeetlah is known to occur within the study area. Table 47, (p. 110) indicates that D. monticola is scarce or absent at the highest elevations in the Unicois, which is where santeetlah reaches its greatest abundance. This suggests that perhaps D. monticola somehow excludes santeetlah from seepages at intermediate elevations. The absence of fuscus at intermediate elevations may also be due to the presence of D. monticola and D. quadramaculatus at these elevations, since both are present at relatively high densities. The nature of the interactions, if they occur, is unclear, but both predation (Hairston, 1980) and competition, including behavioral interactions, (Keen, 1982; Kryzysik, 1979) have been implicated in other assemblages of Desmognathus.

Keen (1982) also showed that both fuscus and D. monticola had clear habitat preferences irrespective of the effects of competitive interactions. The apparent specialization of santeetlah may have arisen as a result of this type of habitat specificity and because of the absence of anything that resembled typical fuscoid habitat in the headwaters of the streams in the Unicois. Desmognathus santeetlah does not normally occur in seepages with extreme gradients nor on rock face seepages in the study area. If fuscus or fuscoid stock was able

to expand into the headwater areas, it would be expected to persist in those habitats most similar to those it occupied at lower elevations; namely, low gradient seepages with relatively little water flow. Further specialization for those habitats would result if these populations became isolated from those at lower elevations. Expansion into other habitats would be prevented by the presence of D. quadramaculatus along streams and by D. ochrophaeus in more terrestrial situations. Selection would then favor even greater specialization for seepage areas.

To obtain answers for some of the questions listed above will require long term monitoring of both santeetlah and fuscus populations. For example, determining whether higher predation pressure or lower environmental predictability is responsible for the higher reproductive output of santeetlah would require long term mark-recapture studies similar to those conducted by Tilley (1977) so that adult survival could be compared in the two species. This could be coupled with environmental monitoring to determine the frequency of droughts and their relationships to nesting success in seepages and along stream-banks. These studies, however, would only supply supportive evidence for the hypotheses. Determining the reasons for the absence of santeetlah and fuscus at intermediate elevations would be more directly testable, either through the removal experiments as proposed by Hairston (1980) or the lab enclosures used by Keen (1982). In both cases, direct tests of the effects of D. monticola and D. quadramaculatus on santeetlah and fuscus populations would be possible.

LITERATURE CITED

LITERATURE CITED

- Bruce, R. C. 1972. Variation in the life cycle of the salamander Gyrinophilus porphyriticus. *Herpetologica* 28:230-245.
- . 1978. Reproductive biology of the salamander Pseudotriton ruber in the southern Blue Ridge Mountains. *Copeia* 1978:417-423.
- Danstedt, R. T., Jr. 1975. Local geographic variation in demographic parameters and body size of Desmognathus fuscus (Amphibia: Plethodontidae). *Ecology* 56:1054-1067.
- Dennis, D. M. 1962. Notes on the nesting habits of Desmognathus fuscus fuscus (Raf.) in Licking County, Ohio. *J. Ohio Herpetol. Soc.* 3:28-35.
- Dunn, E. R. 1926. The Salamanders of the Family Plethodontidae. Smith College, Northampton, Mass.
- Folkerts, G. W. 1968. The genus Desmognathus Baird (Amphibia: Plethodontidae) in Alabama. Unpubl. Ph.D dissertation, Auburn Univ., Auburn, Ala.
- Green, R. H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley & Sons, New York.
- Hall, R. J. 1977. A population analysis of two species of stream-side salamanders, genus Desmognathus. *Herpetologica* 33:109-113.
- Hairston, N. G. 1980. Species packing in the salamander genus Desmognathus: What are the interspecific interactions involved? *Am. Natur.* 115:354-366.
- Houck, L. D. 1977. Reproductive biology of a neotropical salamander, Bolitoglossa rostrata. *Copeia* 1977:70-83.
- Huheey, J. E. and R. A. Brandon. 1973. Rock-face populations of the mountain salamander, Desmognathus ochrophaeus, in North Carolina. *Ecol. Monogr.* 43:59-77.
- Humphrey, R. R. 1922. The multiple testis in urodeles. *Biol. Bull.* 43:45-67.
- Juterbock, J. E. 1978. Sexual dimorphism and maturity characteristics of three species of Desmognathus (Amphibia, Urodela, Plethodontidae). *J. Herpetol.* 12:217-230.

- Keen, W. H. 1982. Habitat selection and interspecific competition in two species of plethodontid salamanders. *Ecology* 63:94-102.
- Klecka, W. R. 1975. Discriminant Analysis. In: H. H. Nie (ed.) *SPSS Manual*, 2nd ed. McGraw-Hill, New York.
- Kleinbaum, D. G. and L. L. Kupper. 1978. *Applied Regression Analysis and Other Multivariable Methods*. Duxbury Press, Belmont, Calif.
- Krzyzysik, A. J. 1979. Resource allocation, coexistence, and the niche structure of a streambank salamander community. *Ecol. Monogr.* 49:173-194.
- Malter, J. L. 1977. The Flora of Citico Creek Wilderness Study Area, Cherokee National Forest, Monroe County, Tennessee. Unpubl. MS Thesis, Univ. Tenn., Knoxville.
- Marynick, S. 1971. Long term storage of sperm in Desmognathus fuscus from Louisiana. *Copeia* 1971:345-347.
- Nagel, J. W. 1977. Life history of the red-backed salamander, Plethodon cinereus, in northeastern Tennessee. *Herpetologica* 33:13-18.
- _____. 1979. Life history of the ravine salamander (Plethodon richmondi) in northeastern Tennessee. *Herpetologica* 35:38-43.
- Noble, G. K. and G. Evans. 1932. Observations and experiments on the life history of the salamander, Desmognathus fuscus fuscus (Rafinesque). *Amer. Mus. Novitates* 533:1-16.
- Organ, J. A. 1961. Studies of the local distribution, life history and population dynamics of the salamander genus Desmognathus in Virginia. *Ecol. Monogr.* 31:189-220.
- Orser, J. A. and D. J. Shure. 1975. Population cycles and activity patterns of the dusky salamander Desmognathus fuscus fuscus. *Am. Midl. Natur.* 93:403-410.
- Pianka, E. R. 1970. On r- and K- selction. *Am. Natur.* 104:592-597.
- SAS Institute, Inc. 1979. *A Users Guide to SAS 79*. SAS Institute Raleigh, N.C.
- Schoener, T. M. 1979. Inferring the properties of predation and other injury-producing agents from injury frequencies. *Ecology* 60:1110-1115.

- Shaffer, H. B. 1978. Relative predation pressure on salamanders (Caudata: Plethodontidae) along an altitudinal transect in Guatemala. *Copeia* 1978:268-272.
- Sokal, R. R. and F. J. Rohlf. 1969. *Biometry*. W. H. Freeman and Company, San Francisco.
- Spight, T. M. 1967. Population structure and biomass production by a stream salamander. *Am. Midl. Natur.* 78:437-447.
- Tanner, J. T. 1978. *Guide to the Study of Animal Populations*. Univ. of Tennessee Press, Knoxville.
- Tilley, S. G. 1968. Size-fecundity relationships and their evolutionary implications in five desmognathine salamanders. *Evolution* 22:806-816.
- _____. 1973a. Life histories and natural selection in populations of the salamander Desmognathus ochrophaeus. *Ecology* 54:3-17.
- _____. 1973b. Observations on the larval period and female reproductive ecology of Desmognathus ochrophaeus (Amphibia: Plethodontidae) in western North Carolina. *Am. Midl. Natur.* 89:394-407.
- _____. 1977. Studies of Life Histories and Reproduction in North American Plethodontid Salamanders, p. 1-41. In: Taylor, D. H. and S. I. Guttman (eds.). *The Reproductive Biology of Amphibians*. Plenum Press, New York.
- _____. 1981. A new species of Desnognathus (Amphibia: Caudata: Plethodontidae) from the southern Appalachian Mountains. *Occ. Papers Mus. Zool. Univ. Mich.* 695:1-23.
- Tilley, S. G. and D. W. Tinkle. 1968. A reinterpretation of the reproductive cycle and demography of the salamander Desmognathus ochrophaeus. *Copeia* 1968:299-303.
- Wake, D. B. 1966. Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. *Memoirs Southern Calif. Acad. Sci.* 4:1-111.
- Wilder, I. L. W. and E. R. Dunn. 1920. The correlation of lunglessness in salamanders with a mountain brook habitat. *Copeia* 84:63-68.

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