

**1999 ANNUAL REPORT SHENANDOAH NATIONAL PARK
INVENTORY AND MONITORING PROGRAM -PRIMENet AMPHIBIAN PROJECT**

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Amphibian declines worldwide have created a need for more extensive and standardized monitoring of amphibian populations and for elucidating underlying causes of amphibian declines (Heyer et al. 1994). In response to concerns about amphibian declines and malformations, a study evaluating and validating amphibian monitoring techniques was initiated in 1998 in Shenandoah National Park under the auspices of PRIMENet (Park Research and Intensive Monitoring of Ecosystems Network), an interagency effort of the EPA and NFS.

A web site describing the study is found at <http://www.mpl-pwrc.usgs.gov/amphib/primenet>. Field work was conducted by four-person field crews from March-October 1998 and from March-August 1999. In general, we documented amphibian (egg, larva, adult) distribution and abundance using various survey methods (e.g., area- and time-constrained searches in plots and along transects, artificial cover objects, leaf litter bags, 1 m² quadrats, chorus surveys). Amphibians were measured and sexed in the field. Deformities or diseases were documented, but in the two years, only two deformed amphibians and no diseased amphibians were found. Population size estimates were assessed for terrestrial and streamside salamanders using mark-recapture (visible implant fluorescent elastomer) and/or removal techniques. We also recorded many environmental variables, including water quality (temperature, pH, acid-neutralizing capacity, conductivity), soil moisture and pH, and habitat (e.g., streambank composition), in order to relate these to variation in amphibian population parameters. We also completed a study of *Plethodon shenandoah* genetics and initiated monitoring methods for this federally-endangered species.

The primary goals of the study were to:

- Evaluate precision, bias, and efficiency of several sampling methods for terrestrial and streamside salamanders
- Conduct validation studies to determine the relationship between capture indices and adjusted population estimates using capture-recapture and removal techniques
- Develop protocols for long-term park-wide monitoring of amphibian populations
- Assess spatial and temporal variation or changes in amphibian populations in relation to environmental variables
- Establish a monitoring program for the federally-endangered Shenandoah salamander (*Plethodon shenandoah*) and conduct population genetics research on this species

Twenty-four amphibian species (14 salamanders, 10 anurans) known historically to be in the Park are listed below (Witt 1993). Distribution and abundance of 17 amphibian species (10 salamanders, 7 anurans; those preceded by an asterisk) were documented in the Park in 1998 and 1999. In 1998 and 1999, museum specimens of 26 individuals representing seven species were collected and accessioned at the National Museum of Natural History in Washington, D.C.

Amphibian Species at Shenandoah National Park

- *Jefferson salamander (*Ambystoma jeffersonianum*)
- *Spotted salamander (*Ambystoma maculatum*)
- *Red-spotted newt (*Notophthalmus v. viridescens*)
- *Northern dusky salamander (*Desmognathus f. fuscus*)
- *Seal salamander (*Desmognathus monticola jeffersoni*)
- *Red-backed salamander (*Plethodon cinereus*)
- *Shenandoah salamander (*Plethodon shenandoah*)
- *White-spotted slimy salamander (*Plethodon cylindraceus*)
- *Four-toed salamander (*Hemidactylium scutatum*)
- *Northern spring salamander (*Gyrinophilus p. porphyriticus*)
- *Eastern mud salamander (*Pseudotriton m. montanus*)
- *Northern red salamander (*Pseudotriton r. ruber*)
- *Northern two-lined salamander (*Eurycea bislineata*)
- *Longtail salamander (*Eurycea l. longicauda*)
- *Eastern American toad (*Bufo a. americanus*)
- *Fowler's toad (*Bufo woodhousii fowleri*)
- *Northern cricket frog (*Acris c. crepitans*)
- *Gray treefrog (*Hyla versicolor*)
- *Northern spring peeper (*Pseudacris c. crucifer*)
- *Upland chorus frog (*Pseudacris triseriata feriarum*)
- *Bullfrog (*Rana catesbeiana*)
- *Green frog (*Rana clamitans melanota*)
- *Wood frog (*Rana sylvatica*)
- *Pickerel frog (*Rana palustris*)

Each of the subsections below represent separate studies within the PRIMENet amphibian and research monitoring program at Shenandoah National Park.

*** Chorus Surveys at Hogcamp Swamp and Big Meadows Swamp**

In the spring of 1999, chorus surveys were conducted at Big Meadows and Hogcamp Swamp. Spring peepers and wood frogs were breeding in both of these areas in March and April 1999. However, by May and June, these areas had dried up, and massive tadpole mortality was observed.

*** Prescribed Burn Effects on Terrestrial Salamanders**

To assess the effects of a prescribed burn on terrestrial salamander populations, we used capture-recapture to estimate population sizes of terrestrial salamanders at paired control-burn plots both before and after a prescribed burn. Our null hypotheses were that the prescribed burn would have no effect on the abundance of red-backed salamanders and no effect on various environmental variables (soil moisture and temperature, vegetation parameters) which could influence the presence or abundance of red-backed salamanders.

Five control plots at Shenks Hollow and five burn plots behind the headquarters at Shenandoah National Park were established in 1998 to monitor salamanders prior to the prescribed burn which took place in April 1999. Both sites are located on the Thornton Gap USGS Quadrangle Topographic Map (Fig. 1). Paired plots (3 paired plots are 15 m², two are 20 m²) were marked with rebar and flagging tape and a round aluminum tag denoting the plot number was affixed to the largest central tree in each plot. In 1998, plots were visited during the day between 1000 and 1800 on four occasions, in the spring (8 April-21 May 1998) and once in the fall (October 1998). In 1999, plots were visited once before the burn (April) and four times after the burn (April, August-October) (Fig. 2). One team searched a control plot while the other team searched the burn plot simultaneously. Plots were split into individual search areas using wire flags (3 m strips) to prevent duplication of search effort. All surface rocks, logs, large twigs, and bark were overturned to find salamanders, and the number of overturned rock and wood cover objects was recorded using clickers. When a salamander was captured, it was immediately placed into a ziploc bag. We blew air into the ziploc bag and sealed it such that the salamander could not escape and was provided with an air cushion to prevent it from getting crushed. We replaced cover objects as carefully as possible, and returned leaf litter and other material around the cover objects if these were displaced. We placed a numbered wire flag in the ground next to the cover object, and wrote the same number using a Sharpie onto the ziploc bag in which the salamander was placed. This allowed field workers to return the salamander to the appropriate cover object after marking. We measured salamanders for snout-vent length and total length and recorded whether the tail was missing or regenerating and whether females were gravid with eggs visible through the abdomen. Red-backed salamander color morph (red or lead) was also recorded.

All red-backed salamanders and white-spotted slimy salamanders greater than 25 mm snout-vent length were marked using visible implant fluorescent elastomer (VIE) (Northwest Marine Technology, Inc.; <http://www.nmt-inc.com>) (Fig. 3). VIE tagging was initially developed for marking fish (Bonneau et al. 1995), but has recently been used successfully to mark amphibians (<http://www.mp1-pwrc.usgs.gov/markings/vie.html>). Elastomer is a latex which, after mixing with a hardener, is a liquid at cold temperatures but cures into a pliable, biocompatible solid at higher temperatures. The elastomer-hardener liquid is placed into a syringe and injected just underneath the skin. We use three colors (C; red, orange, yellow) singly or in combination at any of four locations (L) on the right or left sides of the salamander behind the forelimbs or in front of the hindlimbs (Fig. 3). This marking scheme allows for 255 individual marks ($\{[C + 1]^L\} - 1$). After marking and processing, the salamander was placed next to the cover object such that it could crawl back under the cover object on its own.

Environmental variables recorded during each visit to terrestrial plots included air and soil temperature, wind speed, sky code, soil moisture, pH, and % sand, silt, clay, relative humidity, and leaf litter depth. We also recorded dominant plants in the overstory and understory, % canopy (using spherical densiometer) and % understory cover (in 1 m² areas within plot), and measured diameter at breast height for all trees greater than 5 cm dbh.

Population estimates, detection probabilities, and salamander densities at each of the burn and control plots in 1998 are shown in Table 1. Population estimates were calculated using Program Capture, which assumes a closed population, as tests for closed populations were not violated (Rexstad and Burnham 1991). Various models were used to estimate the populations, and

models differed based on whether capture probabilities were constant (Model O), varied with time (T), by individual (H), by behavioral response to capture (B), or combinations thereof. Red-backed salamander densities in the plots ranged from 0.19-0.98/m², and the number of salamanders per cover object ranged from 0.21-1.88. At any one time, detection probabilities (p , or the proportion of salamanders visible under cover objects) in the plots ranged from 3-31% (mean $\pm$ SD = 0.12 ± 0.099). We found significantly lower densities of (Fig. 4) and smaller (Fig. 5) red-backed salamanders immediately after the burn in the burn plots as compared to the control plots. In spring 1998, coefficients of variation (CV = standard deviation/mean) in counts at the plots ranged from 0.26 to 0.66.

- Artificial Cover Object Study

Artificial cover objects (ACOs), or coverboards, are increasingly being used as a monitoring technique for terrestrial salamanders (<http://www.mpl-pwrc.usgs.gov/sally>). Compared to natural cover objects (NCOs), ACOs provide a more standardized sampling unit, are easier to overturn, may make salamander detection easier, and do not disturb the natural habitat. However, little published information is available comparing capture indices and capture-recapture population estimates under ACOs versus NCOs and how the addition of ACOs might influence salamander density and detectability. Our objective was to compare the use of ACOs to NCOs as a monitoring technique for terrestrial salamanders and to examine the potential influence of adding ACOs to plots on salamander abundance and visibility. Our null hypotheses were that: 1) the addition of ACOs would have no influence on salamander density, 2) ACOs and NCOs would exhibit similar salamander densities (# salamanders/surface area of cover object), and 3) ACOs would not influence the recapture rate of salamanders.

In the fall of 1998, we established eighteen 15 m² plots with six plots in each of 3 areas (Tanner's Ridge, Pocosin Gap, Fisher's Gap), which represent different ages and types of forest stands and previous land use disturbance histories (Fig. 1). We later had to drop four of the plots at Tanner's Ridge. The plots were placed a minimum of 50 m apart. Tanner's Ridge (Big Meadows USGS Quadrangle Topographic Map) currently supports a mixed deciduous forest with a few 70 year old and older trees. This area was cleared for pasture land in 1928 by the early settlers, and a fire occurred here in 1930. In 1941, this area was classified as open grassland with the exception of one small locality of mixed age red oak (*Quercus rubra*). Fisher's Gap (Big Meadows, USGS Quadrangle Topographic Map) was logged prior to 1920 but was never used as pastureland. From 1940's records, the area was described as open yet surrounded by 1-20 year old red oaks. The area borders an area in which a major fire occurred around 1930. Pocosin Ridge (Fletcher USGS Quadrangle Topographic Map) located just south of the Pocosin Fire Road is an older predominately red oak forest (21-40 years old in 1940) that is relatively undisturbed.

Plots were surveyed during the day five times between 23 September and 31 October 1998 and three times in April and May 1999. In late May 1999, we added 36 1 foot" untreated pine boards (ACOs) in a 6 x 6 grid 2 m apart to half the plots at each site. A numbered wire flag was placed next to each ACO and the number was also spray-painted on top of the ACO. After the ACO addition, we surveyed the plots four times monthly in July, August, October, and November. We batch marked salamanders using only one color at one marking position for each visit. On

subsequent visits, recaptured individuals were marked again using the new batch mark. We measured the size of NCOs (length, width, depth) and decay and moisture score for wood cover objects.

After ACO addition, red-backed salamander densities under NCOs were lower in plots with ACOs than in control plots without ACOs (Fig. 6). Additionally, salamander densities under ACOs were much higher than under NCOs in the plots where ACOs were added (Fig. 7). These results suggest that salamanders prefer ACOs to NCOs. We also found significantly higher red-backed salamander recapture rates under ACOs as compared to NCOs in 4 out of 7 plots (Fig. 8). Coefficients of variation (CV = standard deviation/mean) in counts at the plots in the fall of 1998 ranged from 0.25 to 1.24. The CVs were higher for these plots as compared to the control-burn plots in the spring of 1998 because of the drought in the fall of 1998, making salamander counts more variable.

- **Stratified Random Park-Wide Survey of Terrestrial and Streamside Salamanders**

During the summer of 1999, we surveyed amphibians at 48 stratified random terrestrial plots (10 m) and 48 streamside transects (25 x 1 m) throughout the Park. The sites were stratified based on elevation (low = $\leq$ 2,000 ft., high = $>$ 2,000 ft.), bedrock (basaltic, granitic, siliciclastic), and aspect. The data have not yet been analyzed, but we will look at amphibian species richness and abundance at these sites in relation to a suite of environmental variables (environmental measures are described in the other terrestrial and streamside amphibian study descriptions). We will also overlay amphibian data collected from the survey onto GIS maps of vegetation, soil types, and other features to determine relationships between species richness and abundance and other environmental factors.

- **Shenandoah salamander (*Plethodon shenandoah*) Population Genetics and Monitoring**

The Shenandoah salamander was listed as an endangered species by the Commonwealth of Virginia in 1987 and was listed as federally endangered in 1989 (Fig. 9). The principal threats to the salamander are its severely restricted distribution and competition from the closely related red-backed salamander. The principle concerns associated with the restricted range of the species include the potential effects of acid deposition, forest defoliation due to exotic insect species, and potential impacts of roads and trails. A recovery plan in 1994 recommended that standardized long-term monitoring of Shenandoah salamander populations be established (USFWS 1994).

In 1999, we conducted a population genetics study of the three Shenandoah salamander population isolates (Hawksbill, Pinnacles, Stony Man) in the Park. Mitochondrial DNA was extracted and analyzed from tail tips collected from pure Shenandoah salamanders (n = 10 per population) as well as pure red-backed salamanders (n = 10) collected at least 250 m from the edge of the Shenandoah salamander's range. The genetics study, conducted by Dr. Jack Sites and Dan Carpenter at Brigham Young University, Provo, UT., was meant to determine how distinct the three Shenandoah salamander populations were from one another and whether there was evidence for genetic introgression from red-backed salamanders into the core Shenandoah.

salamander areas. Initial results suggest that the three Shenandoah salamander populations are very similar genetically and that there is no evidence for genetic introgression.

Initial surveys revealed that plots may work better than transects for monitoring Shenandoah salamanders. We recommend that plots (10 or 15 m²) be conducted during the day after rains or on days with high relative humidity. Three to six plots should be established for each population, and plots should be checked on a monthly basis in April and May and again in September and October. Surveys should be conducted in a fashion similar to that presented above in the other terrestrial salamander studies.

- **Survey Method Comparison for Streamside Amphibians**

In 1998, we surveyed nine second-order streams for streamside salamanders (Fig. 10). The streams represented a range of water quality (pH, acid-neutralizing capacity) and habitats. Salamanders were surveyed using four techniques: electric shocking, leaf litter refugia bags, 1 m² quadrats, and 50 x 1 m transects (Fig. 11). Our null hypotheses were that: 1) amphibian species richness and abundance would be similar among streams differing in pH and ANC or other water quality variables, and 2) the four survey methods would yield similar species richness and abundance data.

Two 100 m transects for each of nine streams were electric shocked by the fish crews at Shenandoah National Park from June-August 1998. Two amphibian technicians worked behind the fish crews to capture and identify all amphibians encountered during the three-pass electric shocking period. All amphibians were identified to species, measured for snout-vent length and total length, and weighed using a field portable Ohaus scale (200 ± 0.01 g).

We used the leaf litter refugia bag method designed by Pauley (1995). Leaf litter bags made of Deer Block netting, filled with leaf litter and small rocks, and cinched at the top with a cable tie, were placed at 5 m intervals along 100 m transects adjacent to yet upstream of the 100 m electric shocking transects. At each 5 m interval along the 100 m transect; two bags were placed on the right and left sides of the stream, yielding a total of 42 bags per 100 m transect. Leaf litter bags were placed in streams two weeks prior to the first check. The bags were placed at the edge of the stream and surrounded and topped with large rocks to keep them in place. To check leaf litter bags, the rocks were carefully moved away from the bag (noting if any salamanders are under the rocks), and a dipnet was immediately placed underneath the leaf litter bag. The bag was shaken for 10 seconds inside the dipnet and again in a bucket of water. Salamanders that fall into the dipnet or into the water bucket were collected and measured.

At five meter intervals next to the leaf litter bags, we also used streamside 1 m² quadrat sampling (Mitchell 1998). Quadrats were conducted on both sides of the stream, yielding 42 quadrats per 100 m transect. This sampling is destructive, as all stones within a 1 m² quadrat are overturned, and the area is raked. A team of two people turn over rocks and other objects on the right and left sides of the stream along a 50 m x 1 m transect. One person, conducts the survey on the right side and one person works on the left side. Time begin and time end are noted (search usually takes from 30 minutes to 1 hour), and the number of overturned objects is recorded on the data sheet. A clicker assists the observer by counting the number of overturned objects within the 50-

m transect. When salamanders were found, the size of the cover object is recorded. Salamander species, age class, and size class were recorded.

Environmental variables measured at stream sites included water pH, conductivity and acid-neutralizing capacity, stream width and depth, channel type (run, rapid, pool, cascade, riffle, dry), substrate % and type (bank, boulder, large or small rocks, gravel, woody debris, herbaceous cover, shrub, tree, leaf litter).

We found seven salamander and five anuran species along the nine streams. The number of species differed significantly among sites and among survey methods (Fig. 12). The 50 x 1 m transects yielded the highest species richness. The 1 m² quadrat method provided the highest counts of seal salamanders (Fig. 13). For other salamander species, such as two-lined salamanders (Fig. 14), no single method appeared best, though abundance typically differed among streams. An ANCOVA revealed no relationship between two-lined salamander abundance and water pH. Electric shocking was not a good technique for streamside salamanders, which did not float to the surface like fish. The three-pass removal technique did not yield adequate population estimates for streamside salamanders.

• **Intensive Stream Salamander Population Estimate Study**

In 1999, population estimates of streamside salamanders were assessed at six streams using capture-recapture (6 weekly surveys) followed by removal sampling (6 times over 2 days) along 50 x 1 m transects. The total number of salamanders marked in the streams ranged from 52 to 194, and population estimates based on mark-recapture ranged from 81 to 545 (Fig. 15). Recapture rates were low, and probabilities of detection ranged from 1 to 25%. Population estimates using capture-recapture tended to be higher than those from removal estimates (Fig. 15). Coefficients of variation in salamander counts in the streams ranged from 24-70%.

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13:26-35.

SHEN FIGS. PPT

Shenandoah National Park

to go with

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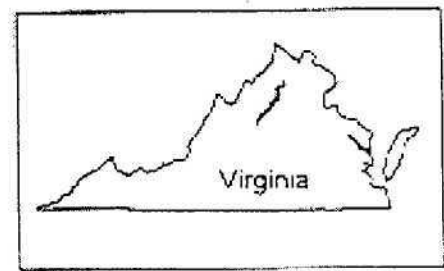
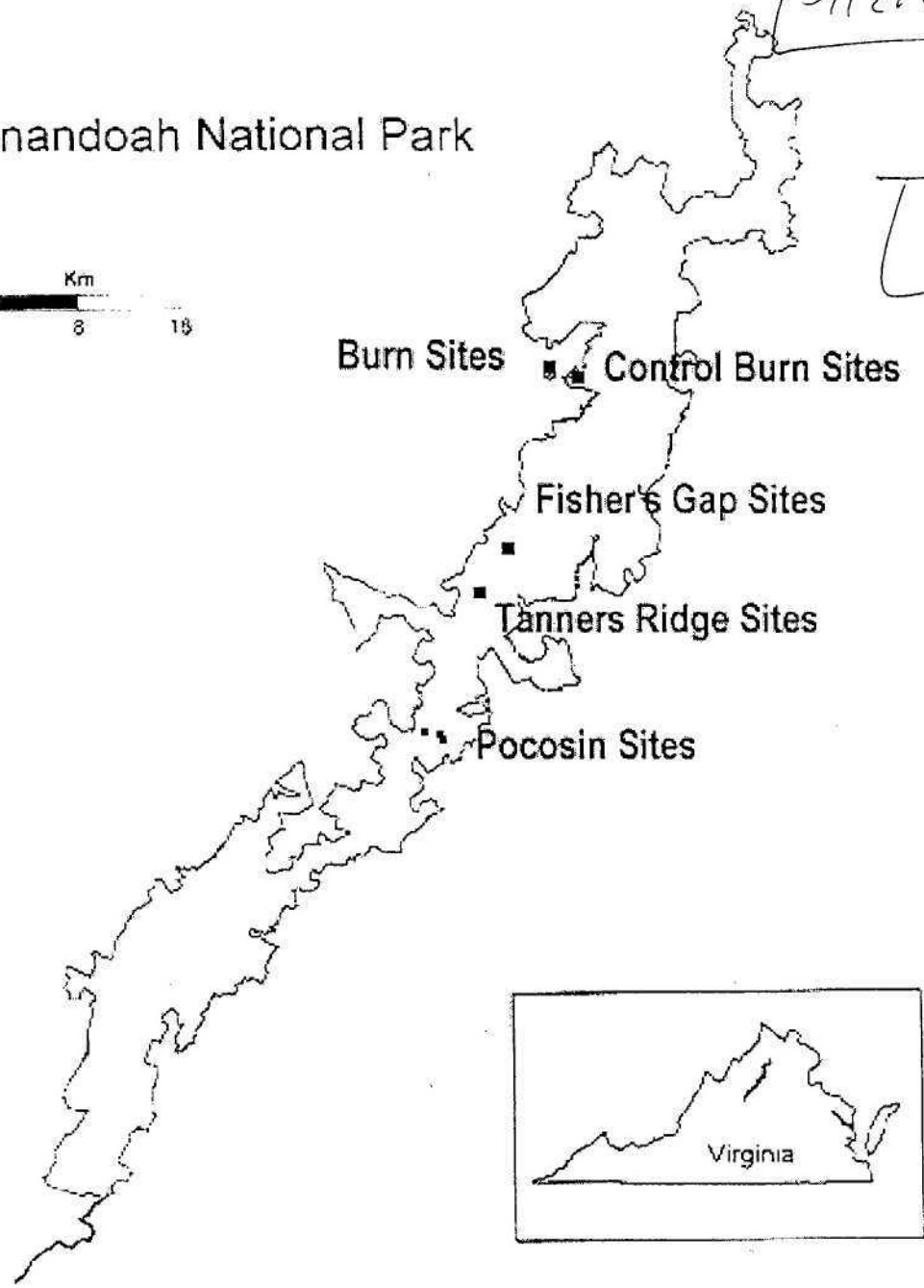
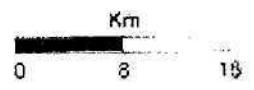
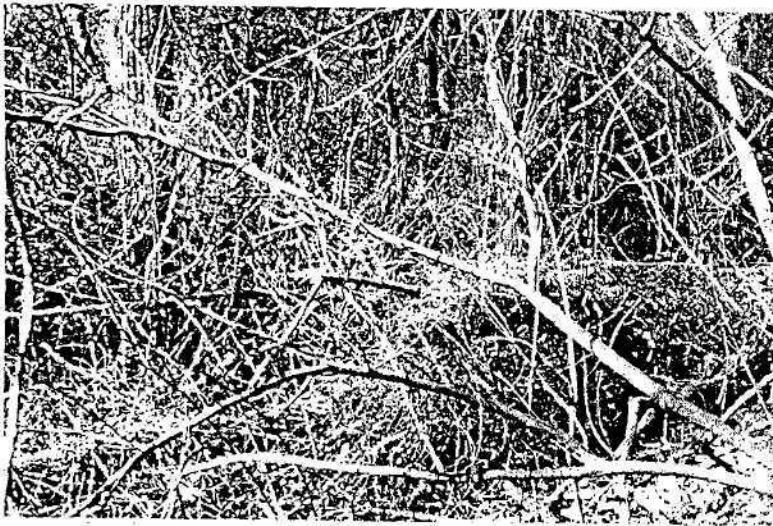
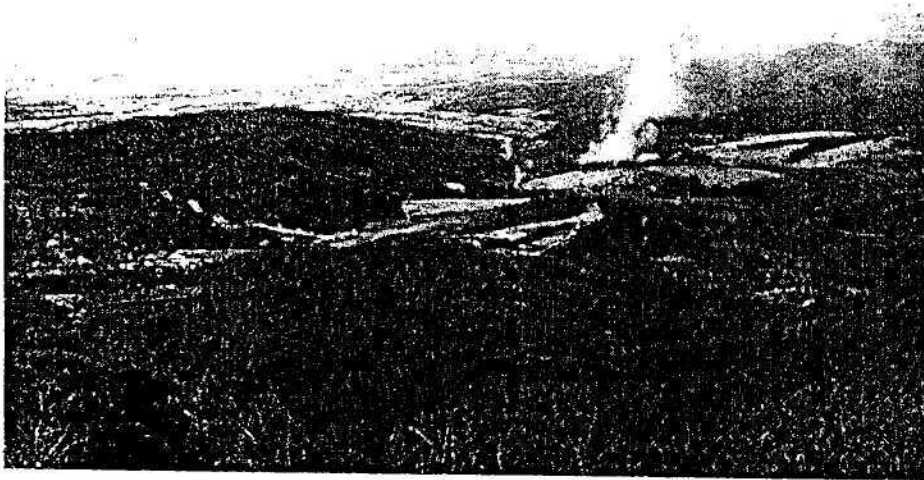
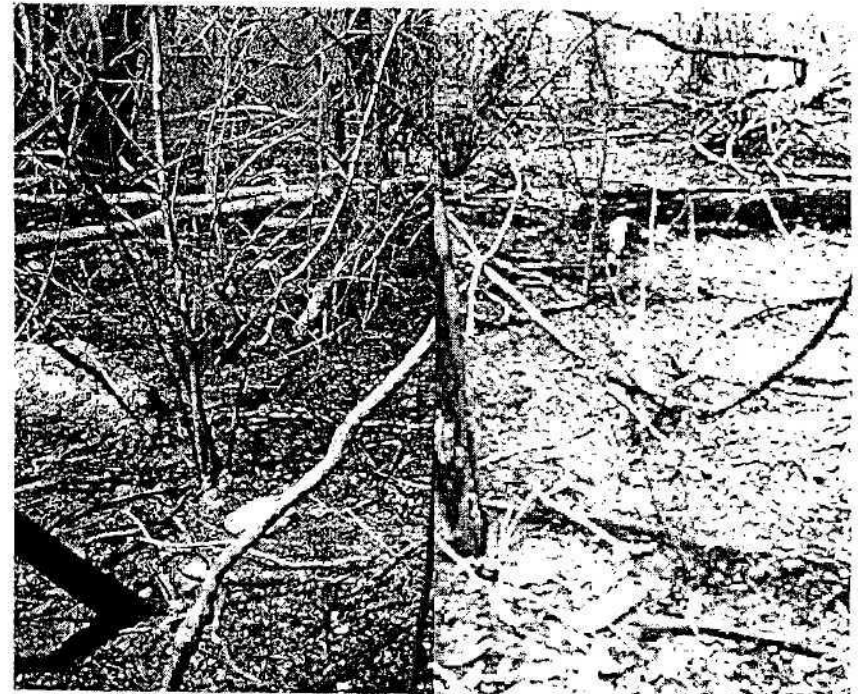


FIG 1

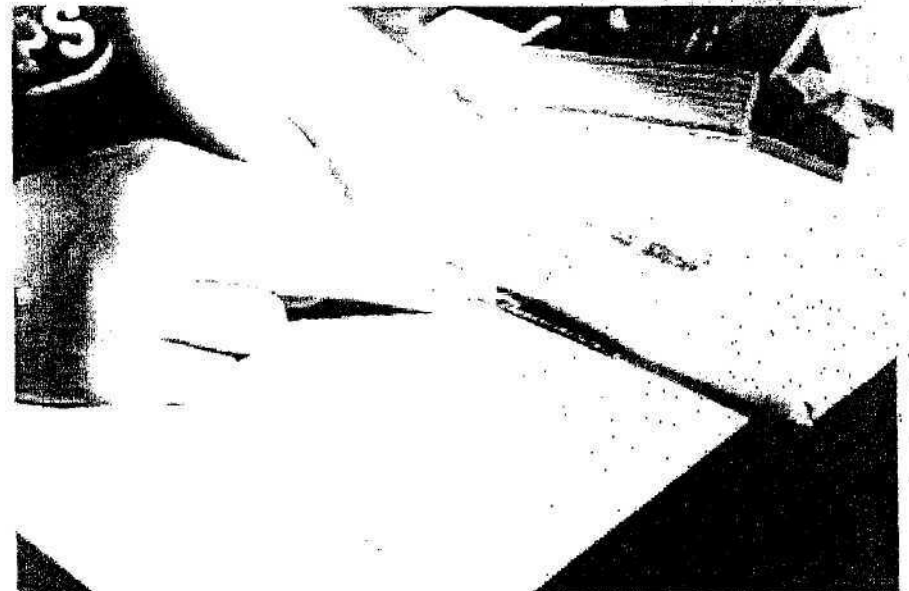
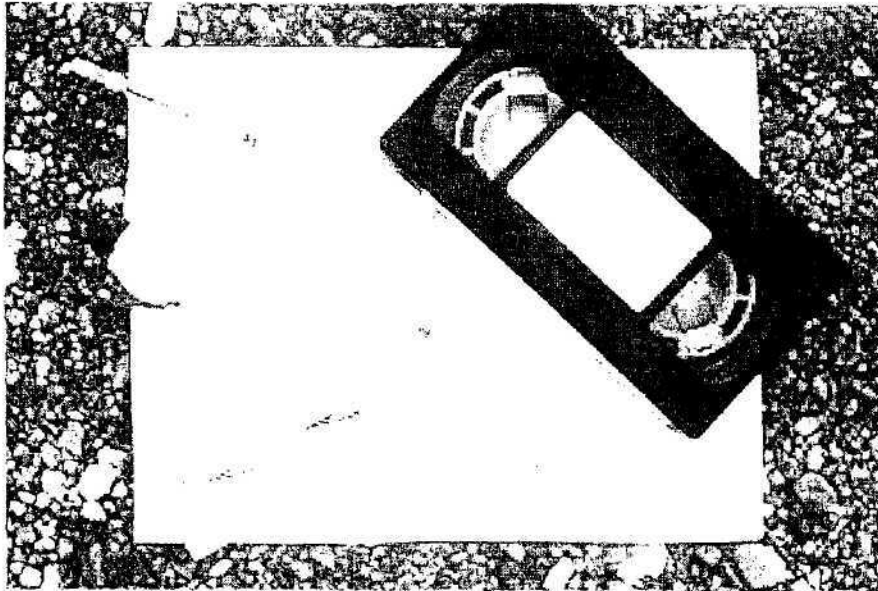


Before Burn



After Burn

(Fig. 2)



Visible Implant fluorescent Elastomer
Northwest Marine Technologies, Inc.

Four positions

Four colors (yellow, red, orange, green)

POPULATION ESTIMATES AND DENSITIES

Redback Salamander (*Plethodon cinereus*) in Shenandoah National Park

Plot	Plot Size (m ²)	# Marked	Proportion Detected	Adjusted Pop ⁿ Size (N)	SE	Model	N/m ²	N/ #CO
C1	1225	138	0.06	646	171	TH	0.53	
C2	400	73	0.12	165	16	H	0.41	0.51
C3	225	42	0.06	199	94	O	0.88	0.30
C5	225	33	0.31	42	9	B	0.19	0.21
C6	225	68	0.25	99	21	B	0.44	0.73
B1	1225	170	0.21	279	52	B	0.23	-
B3	225	63	0.08	220	67	O	0.98	1.62
B4	225	42	0.06	199	94	O	0.88	1.31
B5	400	33	0.03	301	241	O	0.75	1.87
B6	400	26	0.03	282	288	TH	0.71	1.88
F1	225	90	0.08	267	59	O	1.19	0.77
F2	225	43	0.06	162	69	T	0.72	0.39
F3	225	37	0.04	194	127	TB	0.86	0.28
F4	225	99	0.06	359	87	O	1.60	0.95
F5	225	74	0.03	549	257	O	2.44	1.20
F6	225	157	0.13	316	43	T	1.40	0.83
T2	225	81	0.02	900	428	TH	4.00	1.31
T3	225	89	0.03	641	265	T	2.85	1.25
P1	225	93	0.08	251	23	H	1.12	0.67
P2	225	86	0.04	499	186	T	2.22	0.73
P3	225	85	0.19	127	28	BH	0.56	0.20
P4	225	102	0.07	301	69	T	1.34	0.84
P5	225	134	0.05	549	139	T	2.44	1.50
P6	225	172	0.07	535	89	T	2.38	1.14

TABLE 1

Redback Salamander Density in Burn and Control Hots Before and After Burn

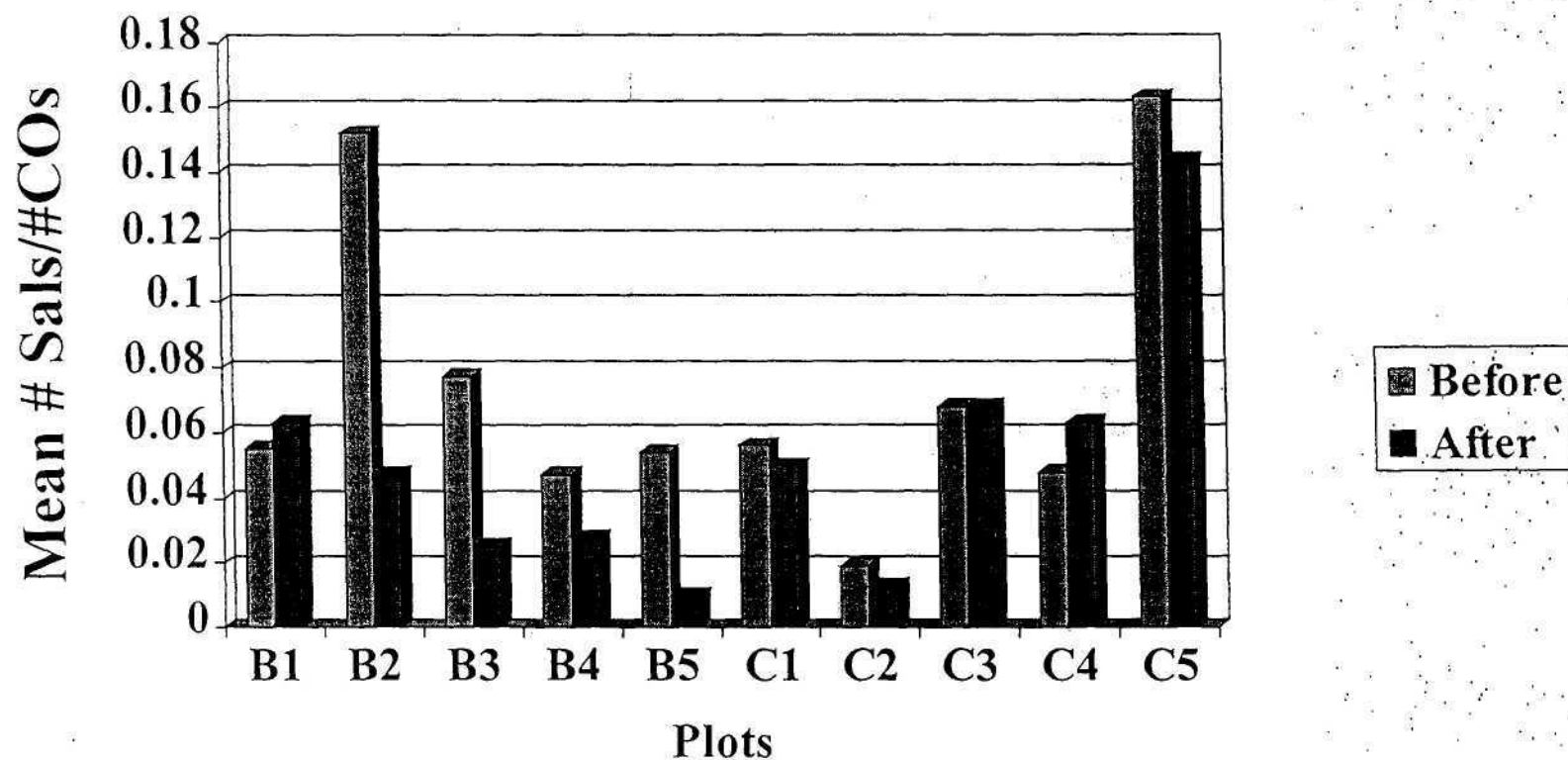


Fig 4

Redback Salamander Snout-vent Length Before and After Burn

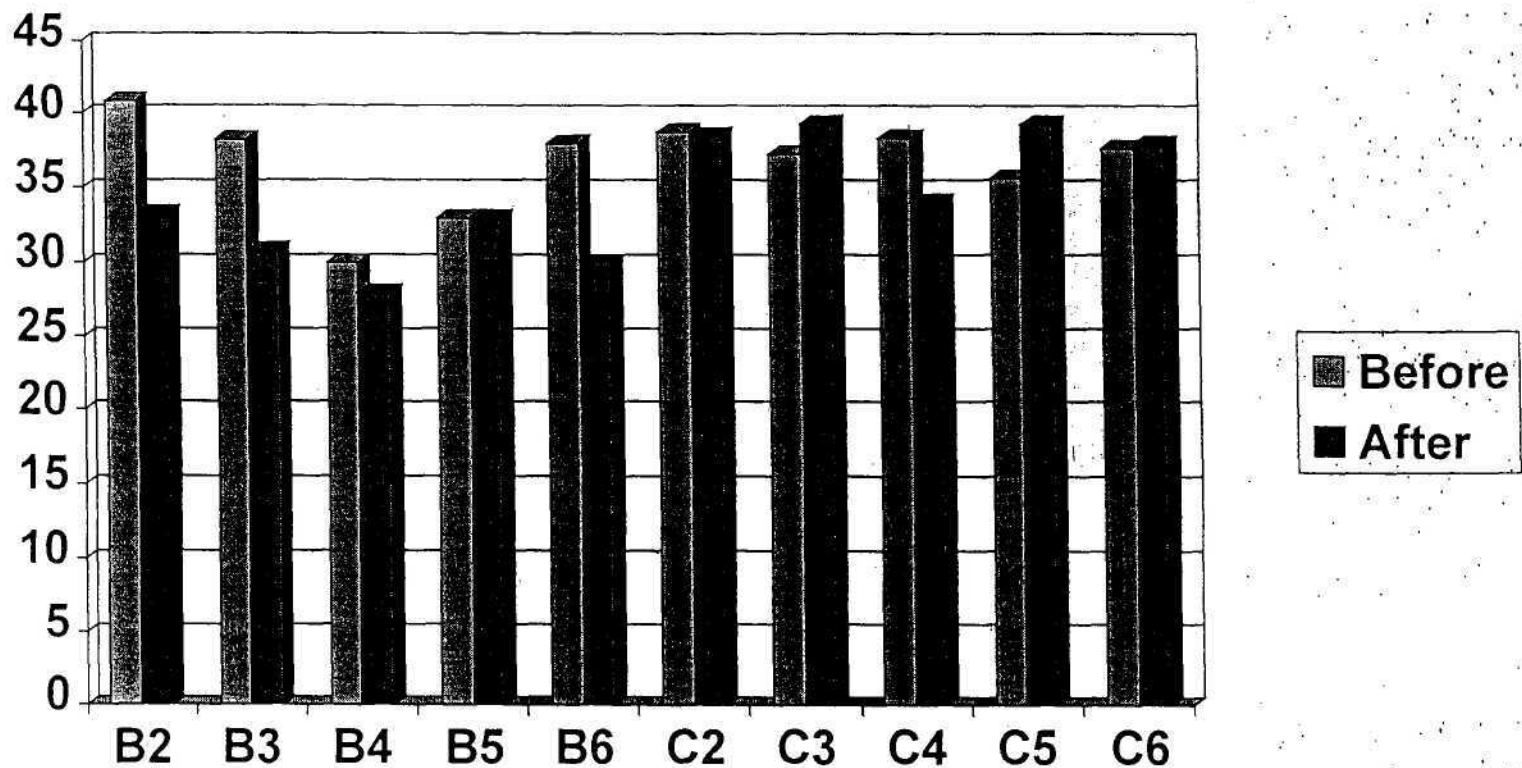


Fig 5

Redback Salamander Density Under NCO Before and After ACO Addition

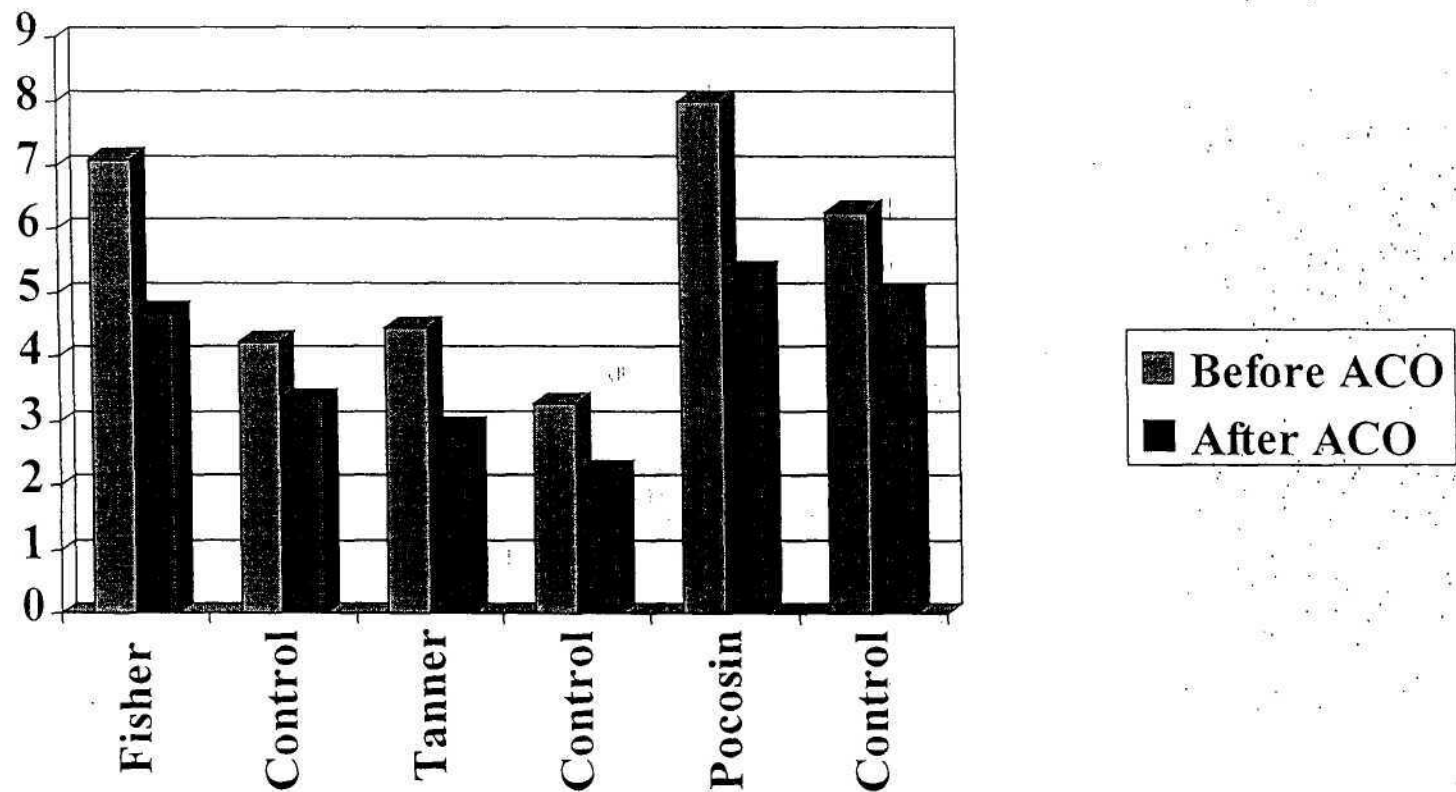
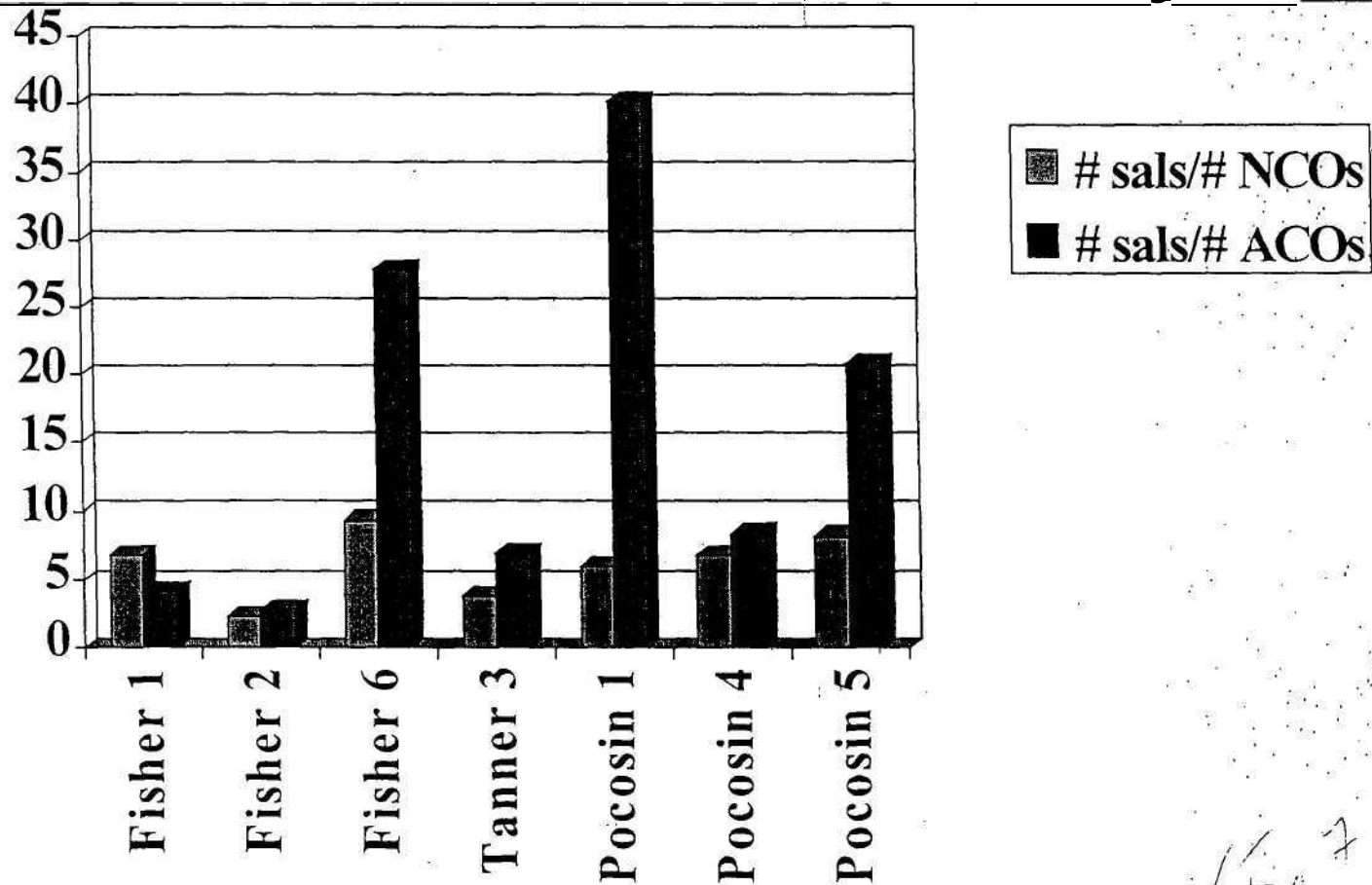


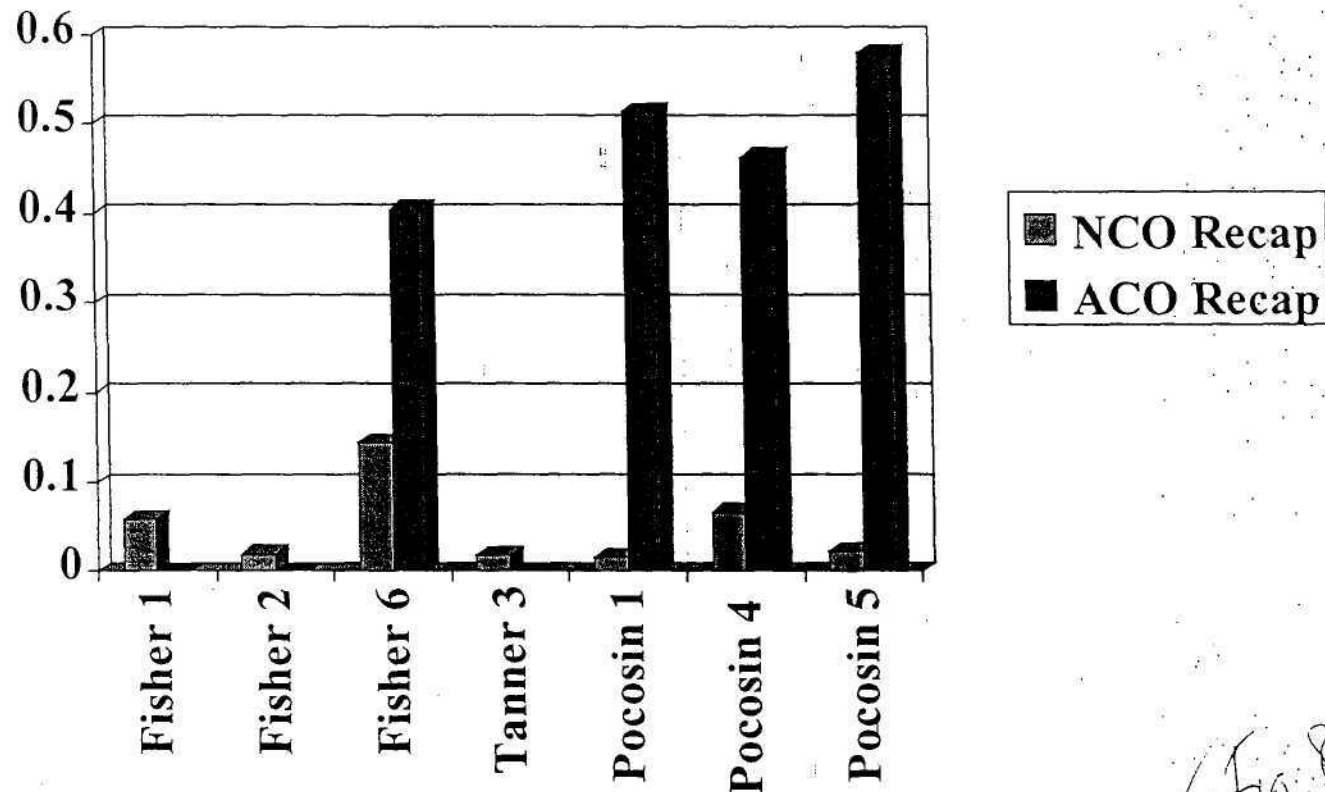
Fig. 6

Redback Salamander Density Under Natural and Artificial Cover Objects

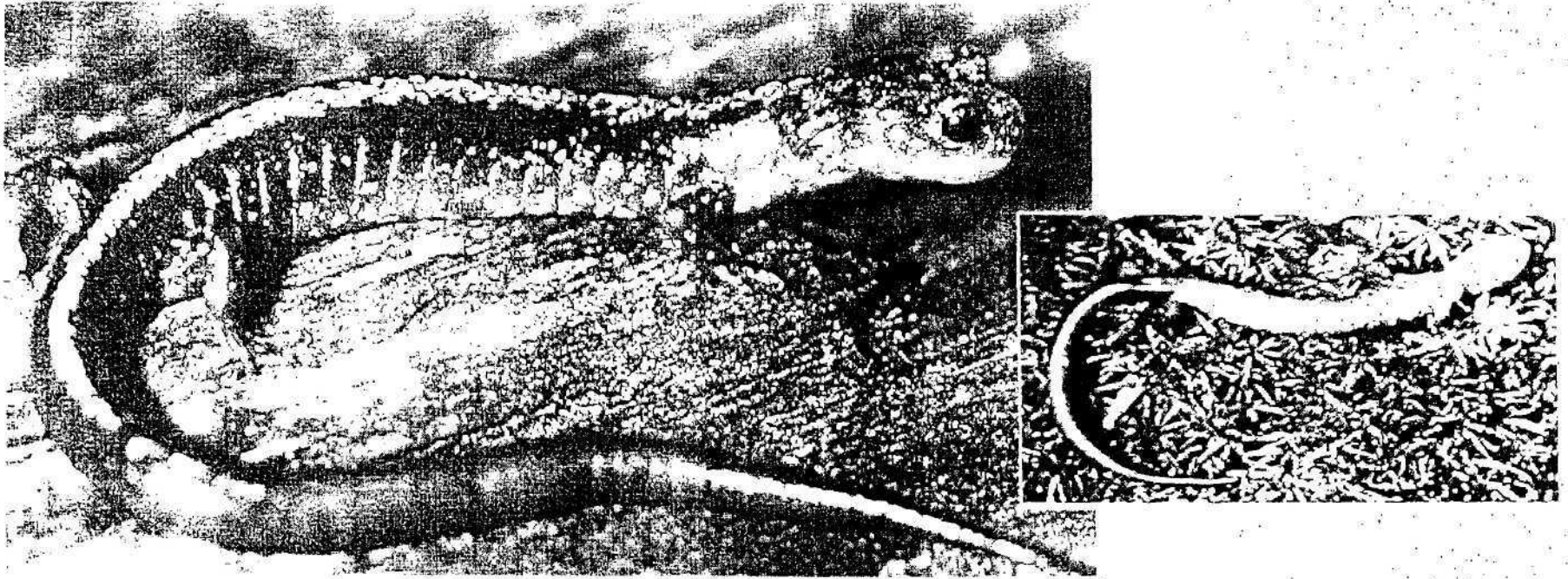


(Page 7)

Adjusted Recapture Rates (# Recaps/# Sals/#COs) Under Natural and Artificial Cover Objects



(Fig 8)



Shenandoah Salamander (*Plethodon shenandoah*)

Federally Endangered Species

Only Found in Three Locations in Shenandoah National Park

One of the Smallest Distributions of Any Terrestrial Vertebrate

Population Genetics

Population Monitoring

Shenandoah National Park

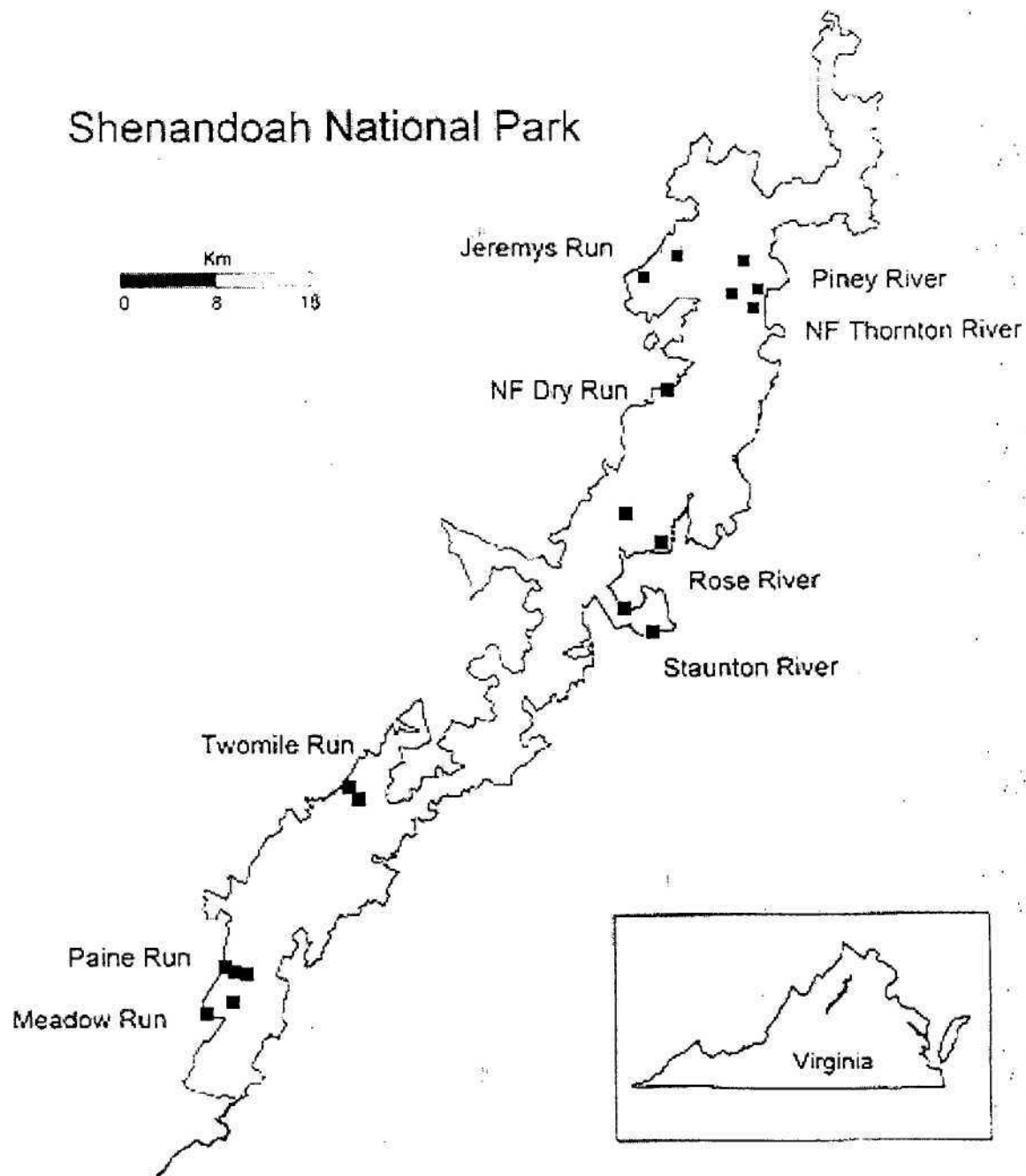
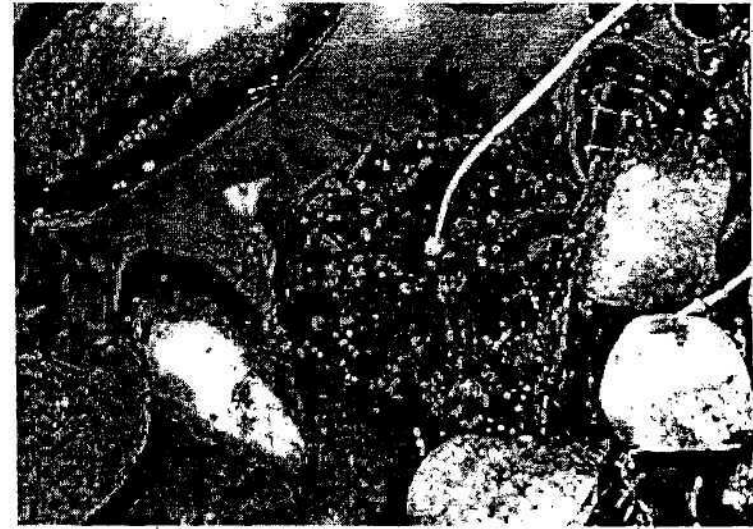


Fig. 10

Streamside Salamander Survey Method Comparison



1 m² Quadrat



Leaf Litter Refugia Bag

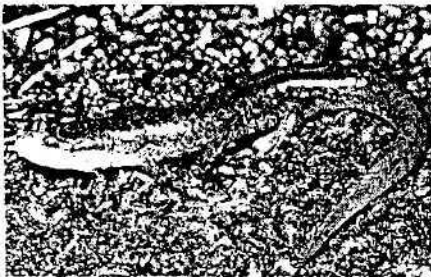


Electric Shocking

**50 x 1 m Visual Encounter
Transect**

Streamside Survey Results

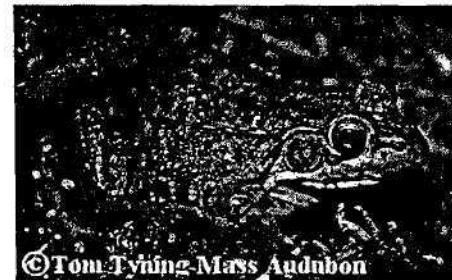
- > Found 7 salamander and 5 anuran species along streams
- > # Species differed significantly among sites and among survey methods
- > 50 x 1 m Transects yielded highest species richness
- > 1 m² Quadrats provided highest counts of Seal Salamander
- > Electric shocking was not a good technique, yet was precise - 3-pass removal did not yield adequate population estimates
- > For other salamander species, no single method appeared best, though abundance typically differed among streams
- > Two-lined Salamander abundance differed among streams but not among survey methods
- > ANCOVA revealed no relationship between Two-Lined Salamander abundance and water pH



Desmognathus fuscus
N. Dusky salamander



Gyrinophilus porphyriticus
Spring salamander



Rana clamitans
Green frog



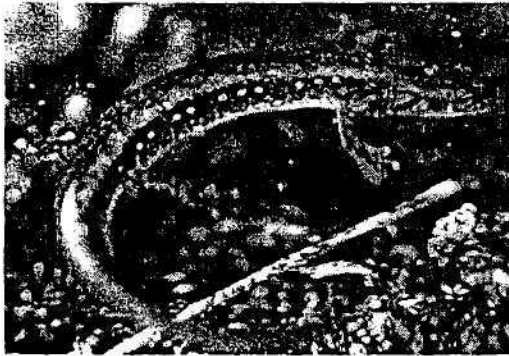
Rana palustris
Pickerel frog

X

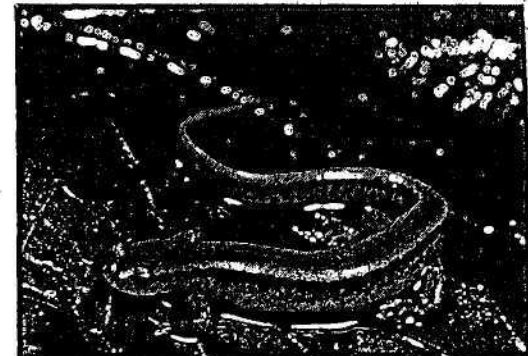
below

* of individuals

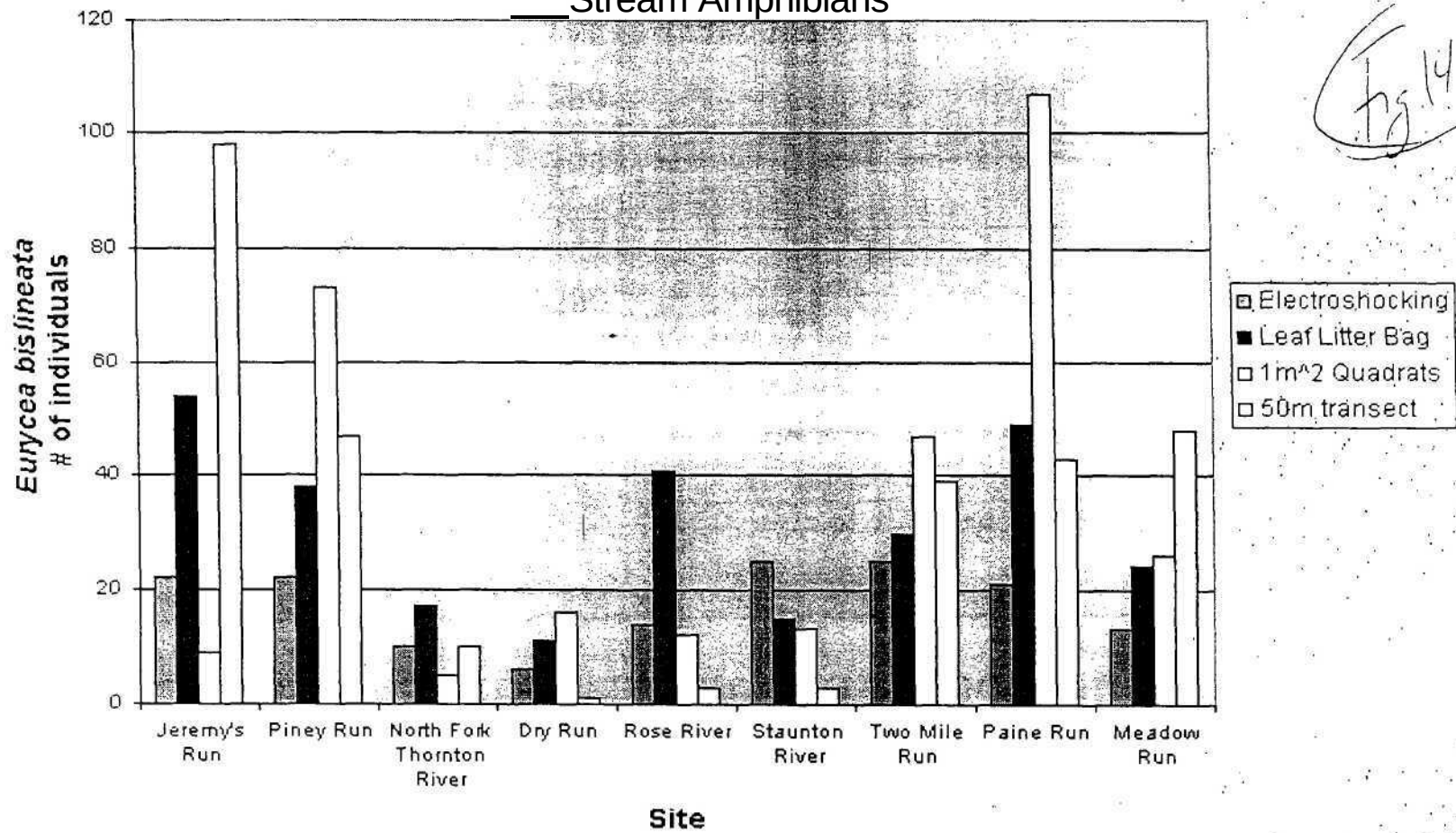
*Desmognathus monticola*



Two-lined Salamander *Eurycea bislineata*



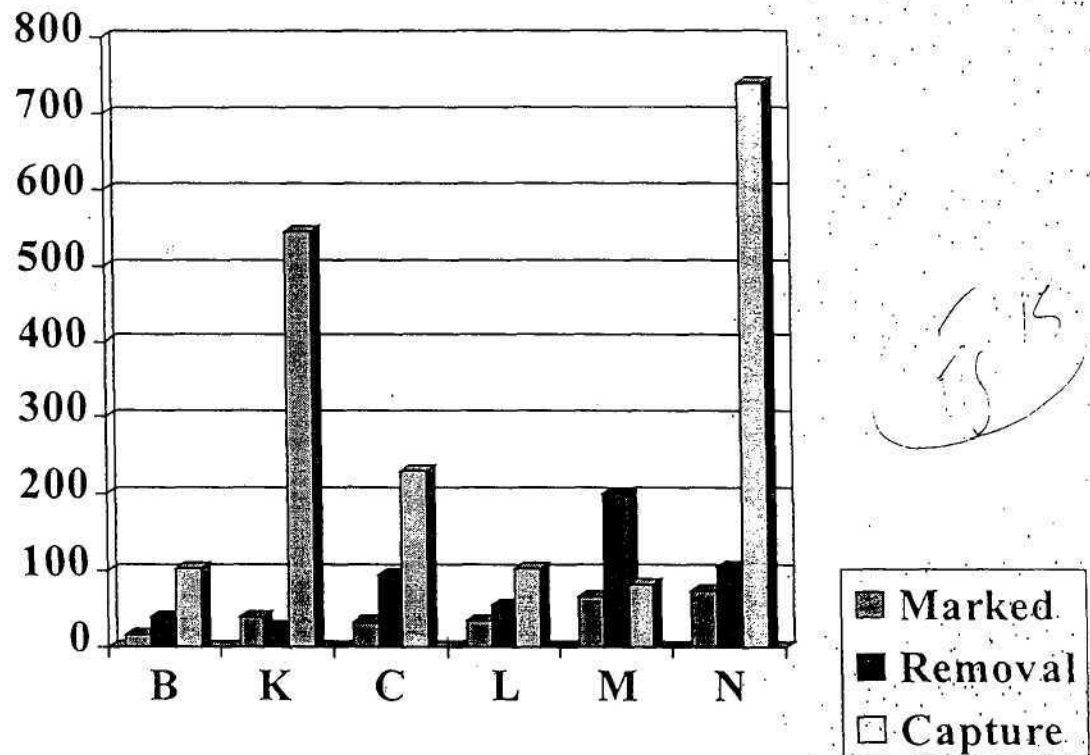
Shenandoah National Park Stream Amphibians



Streamside Salamanders

Counts, Capture-recapture and Removal Estimates

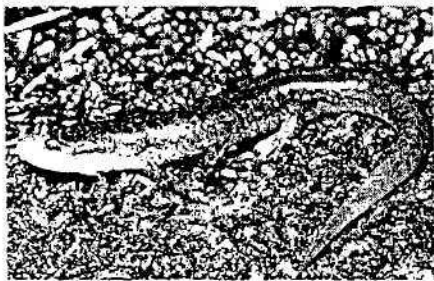
- Six streams
- Six capture-recapture
- Six removals
3/day x 2 days
- Coefficients of Variation in counts
24% - 70%



Streamside Survey Results

- > Found 7 salamander and 5 anuran species along streams
- > # Species differed significantly among sites and among survey methods
- > 50 x 1 m Transects yielded highest species richness
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Fig 12



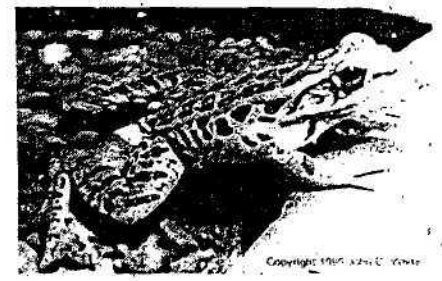
Desmognathus fuscus
N. Dusky salamander



Gyrinophilus porphyriticus
Spring salamander



Rana clamitans
Green frog



Rana palustris
Pickerel frog

X
false

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Fig 12



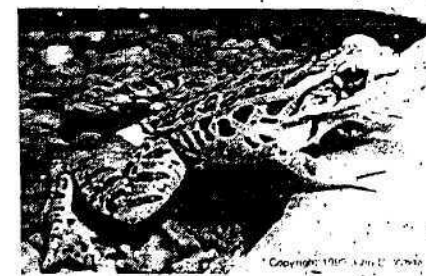
Desmognathus fuscus
N. Dusky salamander



Gyrinophilus porphyriticus
Spring salamander



Rana clamitans
Green frog



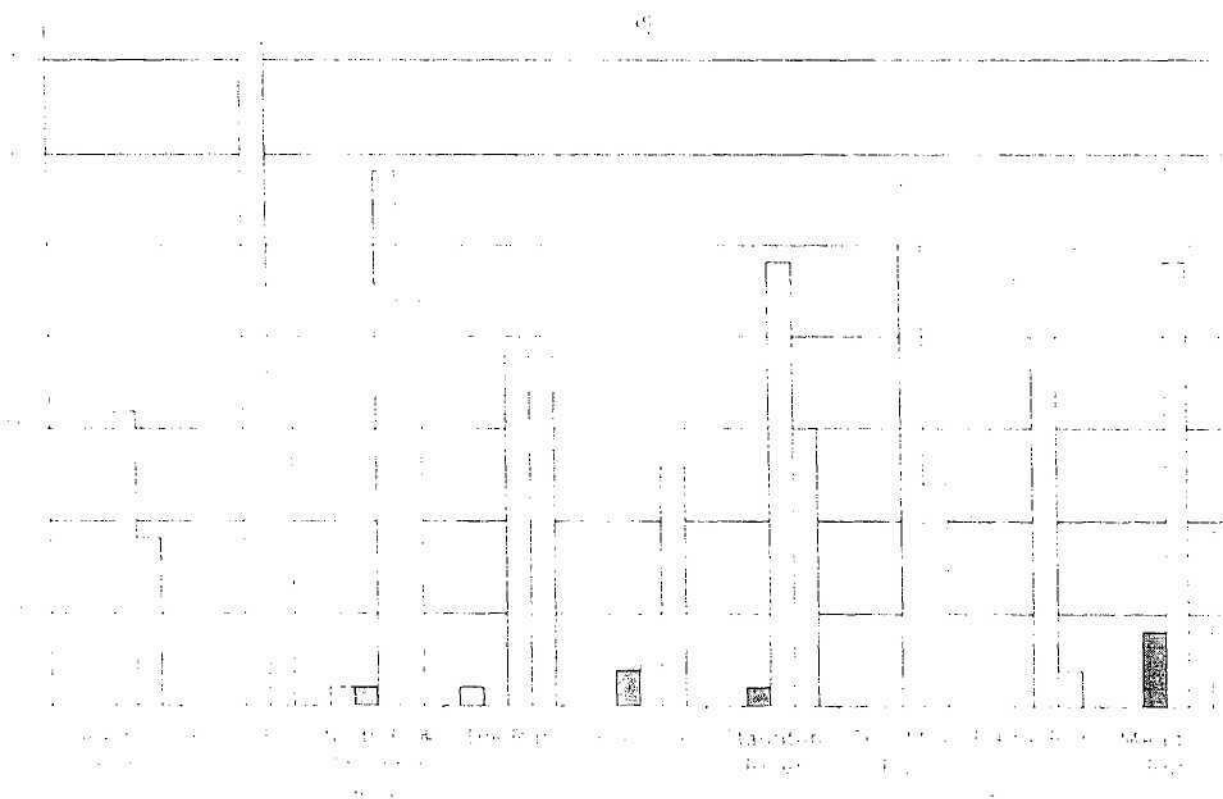
Rana palustris
Pickerel frog

X
to be



Seal Salamander
Desmognathus monticola

Desmognathus monticola
of individuals

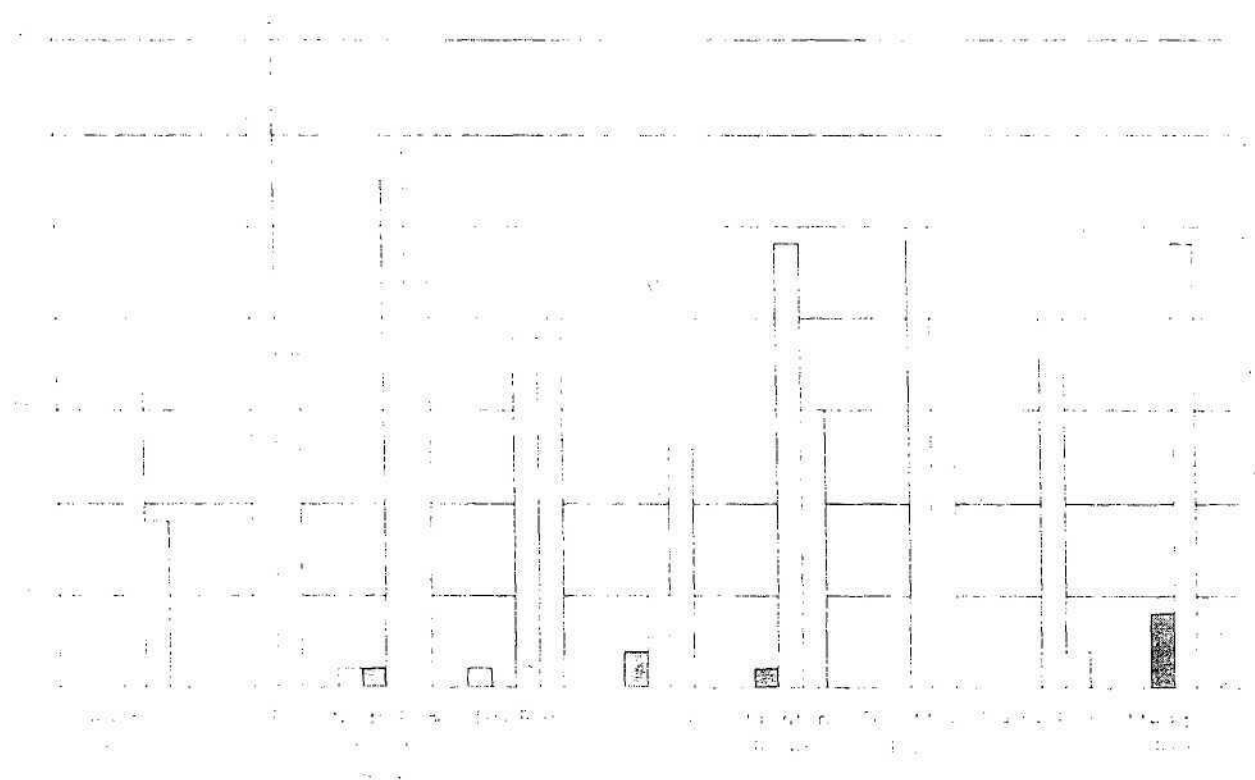


Site



Seal Salamander
Desmognathus monticola

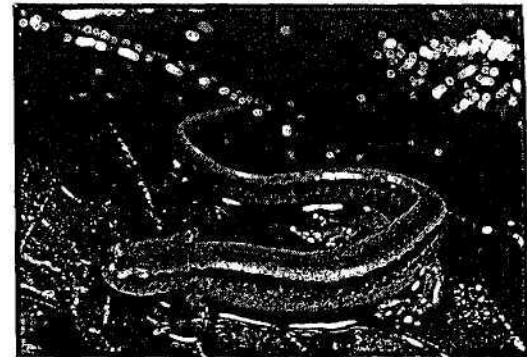
Desmognathus monticola
of individuals



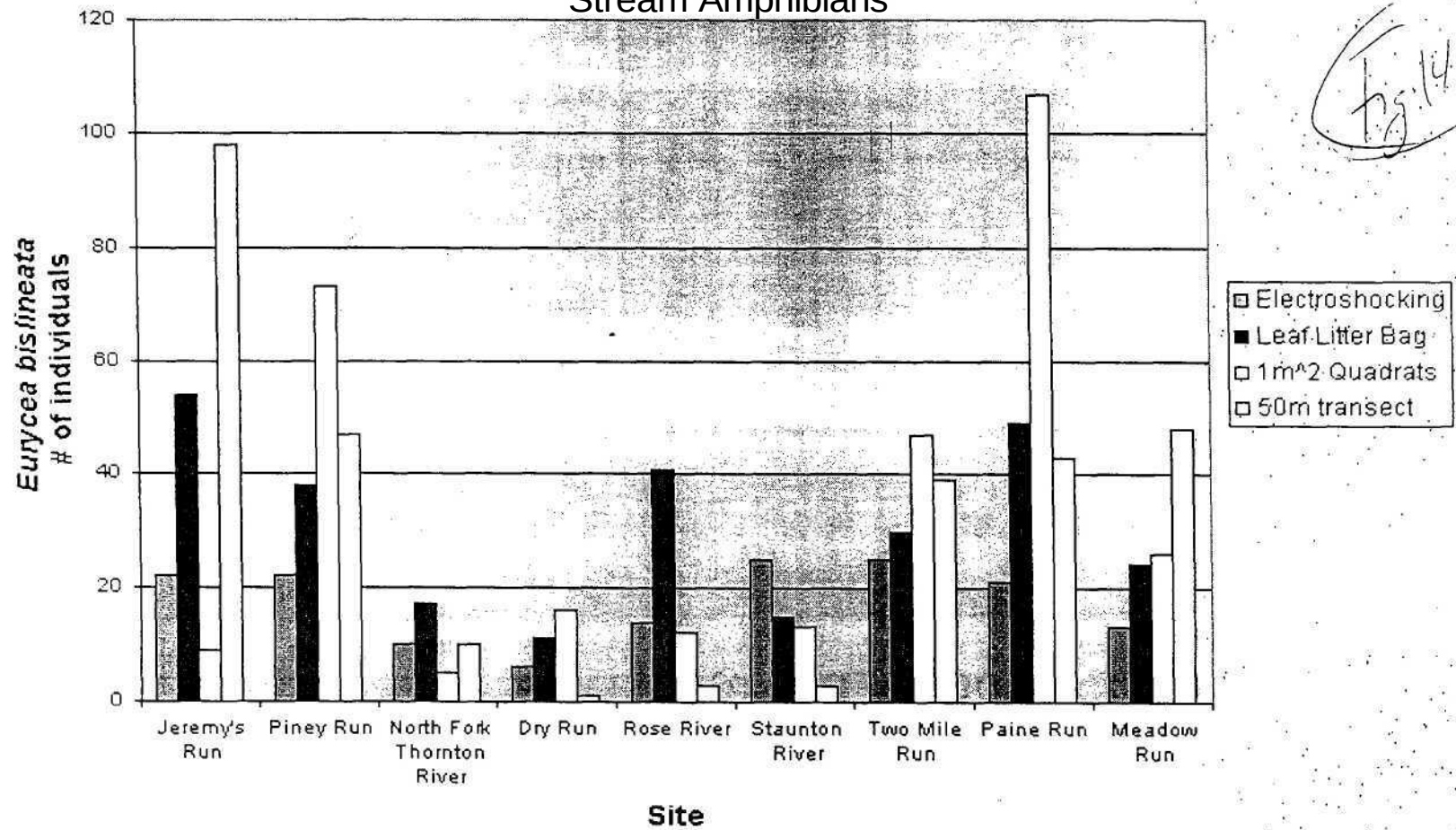
Site

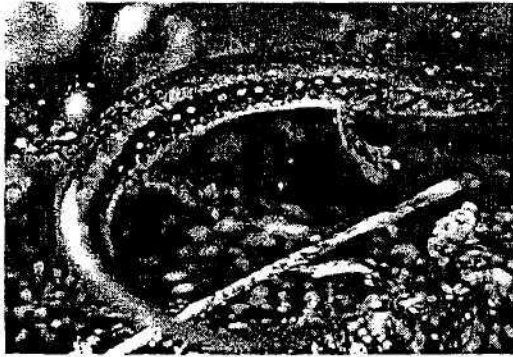


Two-lined Salamander *Eurycea bislineata*

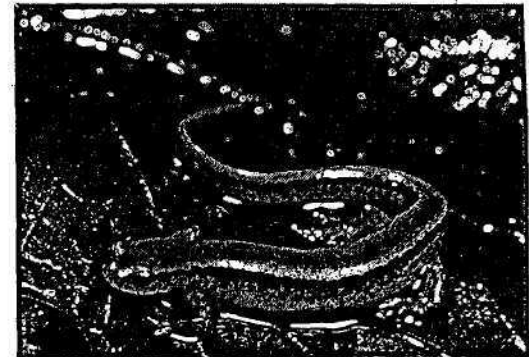


Shenandoah National Park Stream Amphibians

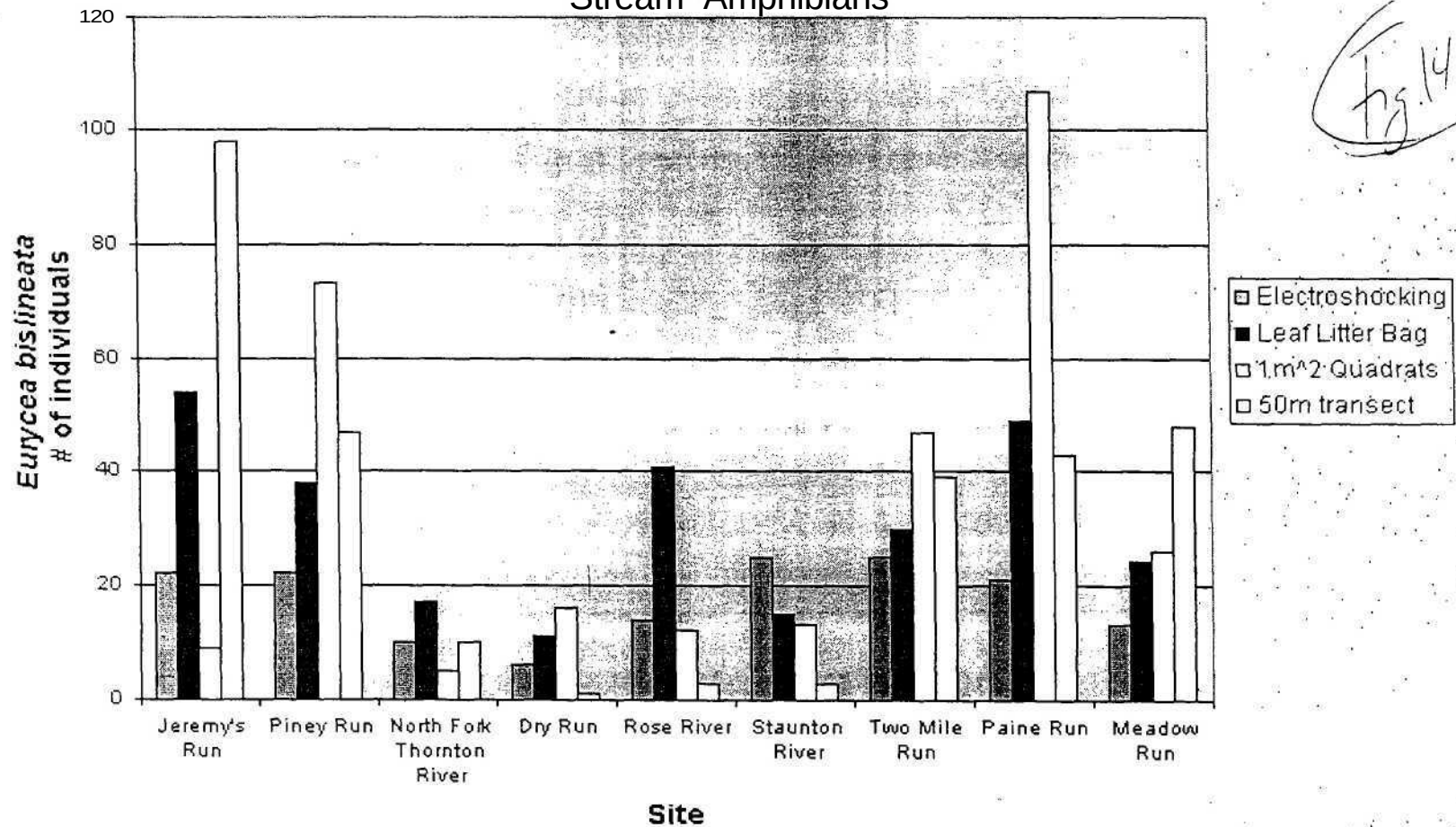




Two-lined Salamander *Eurycea bislineata*



Shenandoah National Park Stream Amphibians



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