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Title: ASPECTS OF THE AUTECOLOGY OF THE PLETHODONTID SALAMANDER, *ANEIDES FERREUS* (COPE)

Abstract approved: Redacted for privacy
Robert M. Storm

Laboratory experiments and simulated field conditions were utilized to gain a better understanding of the ecology of the clouded salamander.

Animals collected in western Oregon were utilized for studies in habitat selection, where it was shown that there was a significant pattern of selection of litter types (rock, bark, and leaves) by size classes and at different temperatures. The young displayed a preference for bark litter, the immature displayed an intermediate preference between rock litter and bark litter, and the adults showed little or no preference between bark litter and rock litter.

Respiratory rates were measured by a Gilson differential respirometer. The effects of size, temperature (10, 20, 25 ^{o}C), and feeding regime were noted. Young animals displayed the highest rates of respiration, with fed individuals significantly higher than nonfed. Immature animals exhibited a respiratory rate intermediate

between the young and adults. Those fed had higher respiratory rates at higher temperatures than did the nonfed. Adults showed the lowest respiratory rates and displayed no significant variation between fed and nonfed individuals.

Population characteristics were described by detailed analysis of the dorsal and ventral color patterns. Individuals were categorized into color classes (adult, immature, and young). Adults tended to have a dark dorsal surface and a relatively light colored venter. Immature animals were more extensively colored on the dorsum with a relatively dark venter. The young displayed the most concentrated colors on the dorsum and had the darkest venter. The live young always possessed blue leucophores.

Reproductive studies revealed the possibility of determining sex, in all sizes of animals, by analysis of the inner vent. Through dissections of animals from all months except January, mature males were shown to have little annual variation in appearance and size of testes and vasa deferentia. Sperm were found in mature male vasa deferentia in every month except September. Males are believed to mature during the second year or at a snout-vent length of over 36 mm. Females contained sperm in their spermathecae in every month that late egg development was evident. Mature females probably lay eggs every other year after attaining a size of about 55 mm (snout-vent length). The first egg laying is probably in the third year of life.

A prolonged breeding period is likely with spermatophore deposition in early May. About 12 eggs are deposited in late June and throughout July with hatching approximately two months later.

The newly hatched individuals measured 25.9 mm (total length) and fed in 14 days.

There was a differential death pattern, inversely proportional to size.

Aspects of the Autecology of the Plethodontid Salamander, *Aneides ferreus* (Cope)

by

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ASPECTS OF THE AUTECOLOGY OF THE PLETHODONTID SALAMANDER, *ANEIDES FERREUS* (COPE)

INTRODUCTION

Studies on the autecology of salamanders are not numerous, but several related to aspects of this investigation have appeared in the literature. In each study emphasis has been placed upon a different set of ecological factors, and a review of these varied approaches should be of value in introducing the present investigation.

Organ (1961) demonstrated in his studies of the population dynamics of the plethodontid salamander, *Desmognathus*, that the more terrestrial the population, the higher the proportion of individuals reaching sexual maturity and the lower the number of eggs produced by each female. A comparison of these findings to the number of eggs produced by a completely terrestrial salamander (*Aneides ferreus*) provides an interesting basis for study.

Stebbins (1954b), in his discussion of the natural history of salamanders of the genus *Ensatina*, utilized marked populations and laboratory animals to study a variety of ecological aspects. He found that female *Ensatina* were capable of storing spermatozoa for at least a month following courtship (spermatophore pick-up) and that there was a slight tendency for larger females to have higher ovarian counts. Throughout his study there was utilization of field investigations and laboratory experiments in a complementary fashion, which is one aspect of the present investigation.

A somewhat similar approach was undertaken by Hendrickson (1954) in his report on the genus Batrachoseps, where he described their ecology and systematics by an analysis of marked populations, color descriptions, regeneration experiments, and growth records. He concluded that at more than 35 mm (snout-vent length) the growth rate became progressively slower, but apparently never ceased completely. Coupled with these data was the observation that individuals were not killed by periods of a few days of freezing temperatures, although their activity was greatly reduced by the consequent lowered metabolism.

No prior investigations have been carried out systematically to determine what affect such lowering (or raising) of temperature has upon a salamander's choice of habitat.

A more general approach to the description of ecological aspects of salamanders was demonstrated through the investigation of two populations of Desmognathus by Rossman (1959). A comparison of size, intensity of pigmentation (color patterns) and ecology resulted in showing no significant differences between the populations considered.

Most ecological observations have been rather limited, since the authors were most interested in the physiology and behavior of a species. Such an approach was the study by Brandon (1965) of the population structure and individual variation in morphology of the salamander Phaeognathus hubrichti. The analysis of reproductive systems, feeding habits of individuals, and the presence of internal parasites were discussed, as was the burrowing activity under laboratory and field conditions.

A detailed experimental study, such as that done by Barbour, Hardin, Schafer and Harvey (1969) on the activity and movements of the dusky salamander (*Desmognathus fuscus*) has not been attempted with the clouded salamander (*Aneides ferreus*). Consequently, the present experimental study was designed to investigate certain ecological factors of this species.

With regard to distribution, the clouded salamander occurs in the humid coastal forests of the Transition Zone from central Mendocino County in California to the Columbia River in Oregon; it is also found on Vancouver Island and certain smaller islands along its eastern coast (Stebbins, 1954a). The distribution in Oregon is limited to the western slopes of the Cascade Mountains and westward to the Pacific Coast. It appears to be entirely absent in the extreme northwest section of the state (Clatsop County) (Stebbins, 1966).

Aneides ferreus is occasionally found under objects on the ground (boards, paper, or wet cloth) but is most readily collected by removing the bark from Douglas fir, Port Orford cedar, redwood, alder, and other logs or by sifting through accumulated leaf litter on the top of sawed stumps of such trees (Stebbins, 1954a). The local habitat preference is in the stumps and fire-charred logs of Douglas fir located in open stands and logged-off areas. Some individuals are found in loose outcroppings of shale-like rock, usually in association with forest clearings or road cut-banks. The animals utilize cracks and tunnels of the inner wood of decayed logs or work themselves into deep, moist cracks of loose rock.

The influence of litter type upon habitat selection, measurement of oxygen consumption (i. e., respiration), and population characteristics with regard to the ecology of a single species have not received detailed attention and therefore emphasis in this study has been placed on the following laboratory investigations.

Habitat Preference

Experiments were designed to answer the basic question of whether there is a selective preference of habitat types available to *Aneides ferreus* in nature or if selection of the type of habitat is merely by a chance movement into the most available source. Gordon (1952), in a detailed study of *Aneides aeneus*, demonstrated that animals showed a definite preference for a habitat consisting of two types of rock crevices, one used for breeding and the other used in a transitory manner.

Heatwole (1962) tested the effect of the physical structure of the habitat (litter types) on *Plethodon cinereus* with results indicating a selective preference for deciduous and conifer litter. Some of the techniques he reported have been modified for this investigation, while others originated with this study.

Respiration

The literature on salamander respiration is not extensive. Evans (1939) tested various factors influencing the oxygen consumption of several species of eastern plethodontid salamanders. She was able to correlate distinct species differences with the activity of the animals. Much later, Vernberg (1952) observed seasonal variations in oxygen consumption of two species of salamanders, noting no significant difference in results of experiments comparing the oxygen consumption of laboratory and field animals. A number of investigations have shown that there is a decrease in metabolic rate with increasing body size (Norris, Grandy and Davis, 1963). Six species of Ambystomatidae (lunged salamanders) have been investigated by Whitford and Hutchison (1963, 1965a, and 1966) in relation to pulmonary oxygen uptake. They related the relative head width to respiratory characteristics and discussed the effect of photoperiod on pulmonary and cutaneous respiration in another work (1965b). Several other poikilothermous animals have been utilized in similar metabolic studies; for example, the effect of starvation upon the metabolic rate of fiddler crabs (Uca) was analyzed by Vernberg (1959a, b). He found that starvation caused an initial decrease in metabolic rate followed by a relatively constant rate. Two species of iguanid lizards were studied by Dawson and Bartholomew (1956) to determine the relation of oxygen consumption to body weight, temperature, and temperature acclimation, while one of these lizards, Uta stansburiana, was studied further by Roberts (1968).

The study of oxygen consumption (i.e., respiration) as related to size class, feeding regime, and temperature has been undertaken for the purpose of providing a better knowledge of Aneides ferreus under conditions which are assumed to be not greatly unlike those encountered by the animals in nature.

Population Composition

Population characteristics of a species can be described by a multitude of approaches, as illustrated by the works of Bogart (1952), Deevey (1947), Hairston (1951), Noble (1930), Pope (1950), and Turner (1960).

In addition to body measurements (snout-vent, tail length) and survivorship records, the following characteristics were selected as a basis for description in the present investigation.

Color Patterns

The literature is extensive with regard to means of describing color patterns of salamanders. In the description of two salamanders of the genus Plethodon, Mittleman (1951) utilized colors and patterns to set apart species, while descriptive categories of dorsal pigmentation and lateral white spotting were developed by Schwartz (1957). Reynolds (1959) described color patterns in *Plethodon jordani jordani* by means of percentage of color coverage of various body areas and Gordon (1960) emphasized the occurrence of brassy specks in discussing color patterns in *Plethodon jordani melaventris*. Other papers dealing with color descriptions in salamanders are those of Highton (1959), Howell and Hawkins (1954), Newman (1954), and Thurow (1955).

In none of the above mentioned investigations were analyses made in an attempt to describe dorsal and ventral color patterns to the degree that has been attempted in the present study, and therefore, most methods used were developed within the course of this investigation.

Reproductive Studies

Reproductive ecology of salamanders has been an active area of investigation. An excellent paper dealing with this subject was published by Sayler (1966) in which are presented several methods which have been modified for this dissertation.

Several valuable investigations have been undertaken by Highton (1956, 1959, 1962) and Highton and Savage (1961). They have presented descriptions of egg laying, animal color phases, time of sexual maturity, and growth patterns of several plethodontid salamanders of the east and southeastern United States.

Organ (1958, 1960a, b) has approached the problem of reproductive ecology by discussions of courtship, breeding behavior, and spermatophores of several species of Plethodon. Different size classes of Eurycea bislineata rivicola were analyzed in relation to growth patterns and sex ratios. It was found that most individuals metamorphose from larva to adult during the third summer or at a size range (snout-vent length) of 23 to 32 mm (Duellman and Wood, 1954).

More specifically related to the present study was the report by Storm (1947) on the eggs and young of Aneides ferreus and a more complete review of the reproductive cycle by the same author in 1948.

Finally, an ecological problem can be approached by the use of field methods or by a laboratory investigation which attempts to simulate field conditions. The latter approach has been employed in this study with the knowledge that the results gained can be utilized for a better understanding of the animals under completely natural conditions.

MATERIALS AND METHODS

Maintenance and Care of Animals

Animals were maintained in covered 12 x 17 cm polystyrene boxes; the bottoms were covered with a folded paper towel and wetted with distilled water. The animals were fed vestigial-winged *Drosophila* twice per week and the paper towels were changed once a week.

The number of individuals per box varied according to the type of experiment being conducted, but no more than 12 animals were ever placed together. All animals were housed in a darkened General Electric incubator (Model 805) at $15^{\circ}C$.

Collecting Locations

Animals utilized in this study were collected in and adjacent to the Willamette Valley (Benton, Lane, and Linn Counties), in the southern coastal region (Curry and Josephine Counties), and in the North Umpqua drainage (Douglas County).

In Table 1, animals are listed chronologically by collecting date, in four groups (I, IIA, IIB, and III). The reason for this organization is that habitat selection experiments, respiration studies, and analysis of color patterns were carried out as specimens became available. In analyzing the accumulated data it became convenient to refer to the

Table 1. Collecting records of live Aneides ferreus used in the study. Experimental classes designated as: Group I (urban habitat of boards, rock, and wet rug); Group II A (rock); Group II B (bark and boards); Group III (mixed bark and rock). All animals were from Oregon locations.

Date of collection	Collecting Site
	Group I
27 Feb. 1968	2252 Willamette Ave., Eugene, Lane County
	Group II A
8 April 1967	1 mile up Road 121, Corvallis Watershed, Benton Co.
4 May 1968	Junction of Canyon Cr. and Steamboat Cr., Douglas Co.
4 May 1968	Bogus Cr., Douglas Co.
17 March 1969	7 miles South Gold Beach; Cape Sebastian, Curry Co.
18 March 1969	Junction Roads 4013 and 3907; Winchuck Forest Camp, Curry Co.
18 March 1969	5 miles South on China Cr. Road, Curry Co.
2 April 1969	1.3 miles up Highway 46 from U.S. 199; Grayback Camp, Josephine Co.
8 April 1969	Middle Fork of Rock Cr. Road 121, Benton Co.
18 April 1969	2.5 miles up Road 121, Corvallis Watershed, Benton Co.
	Group II B
18 October 1967	Elk City, Lincoln Co.
14 January 1968	7 miles SW Philomath, Old Peak Road, Benton Co.
24 January 1968	Elmira, Lane Co.
4 May 1968	Junction of Canyon Cr. and Steamboat Cr., Douglas Co.
4 May 1968	Bogus Cr., Douglas Co.
6 October 1968	5 miles SW Philomath, Larson's Mill, Benton Co.
13 October 1968	10 miles West of Junction City, Lane Co.
16 October 1968	BLM Road #13-66, Alsea, Benton Co.
17 October 1968	5 miles SW Philomath, Larson's Mill, Benton Co.
5 January 1969	5 miles SW Philomath, Larson's Mill, Benton Co.
17 March 1969	7 miles South Gold Beach, Cape Sebastian, Curry Co.
18 March 1969	Junction Roads 4013 and 3007, Winchuck Forest Camp, Curry Co.
18 March 1969	5 miles South on China Cr. Road, Curry Co.
2 April 1969	1.3 miles up Highway 46 from U.S. 199; Grayback Camp, Josephine Co.
18 April 1969	2.5 miles up Road 121; Corvallis Watershed, Benton Co.
	Group III
3 May 1969	2 miles up Soda Fork Road from Highway 20, Linn Co.
3 May 1969	1 mile above Green Peter Dam, Linn Co.

animals by these group designations.

Habitat Preference Studies

This series of experiments was conducted during the summer and fall of 1968 and the winter and spring of 1969. The purpose was to test adult, immature and young animals (Table 2) for a selective preference of the following three habitat litter types: flatrock, bark, and leaves, in an attempt to relate the patterns of choice to the natural habitats where the animals were collected.

Table 2. Size classes of *Aneides ferreus* used in the study.

Size class	Mean length (S-V) (mm $\pm$ SD)	Weight range (g)
Adult	58.21 $\pm$ 4.80	2.01 - 5.40
Immature	41.32 $\pm$ 4.69	0.44 - 2.00
Young	23.98 $\pm$ 3.84	0.09 - 0.43

A 64.5 x 47 cm wooden box, coated with fiberglass, was divided into five sections, each approximately 13 cm wide x 47 cm long. The floor of the box was covered with paper towels and wetted with distilled water. The towels were replaced after each trial to insure the non-accumulation of litter and/or olfactory cues. Moist litter was added to the three center sections to approximately three cm depth (Figure 1A). To insure a constant release substratum a 12 x 17 cm polystyrene box lid was used to introduce the trial animals into the habitat

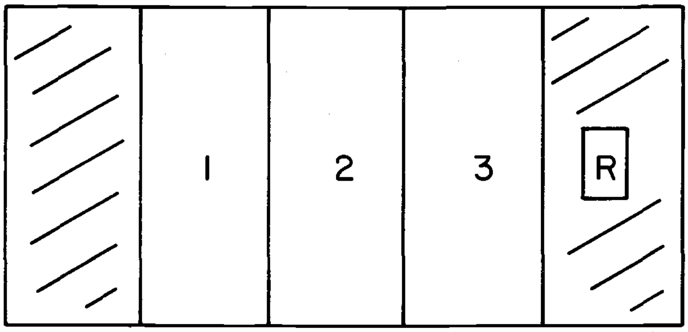


Figure 1A. Habitat selection box showing two blank release points (end sections), releasing lid (R), and three litter sections of flatrock (1), bark (2), and leaves (3).

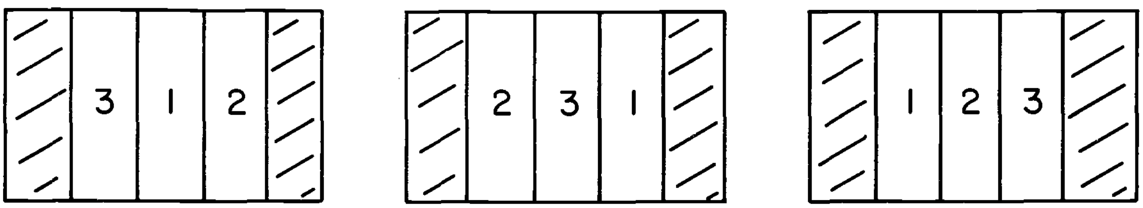


Figure 1B. The design of an experimental habitat selection trial showing sequential rotation of litter types: flatrock (1), bark (2), and leaves (3).

selection box. The releasing position was rotated after each trial.

Figure 1B illustrates the physical set-up, such that between each trial the flatrock, bark, and leaves were removed and rotated in position to eliminate bias resulting from factors different from the selection of habitat types (Heatwole, 1962).

The fiberglass box was tightly covered with a solid glass lid whereby the relative humidity, temperature, and light conditions were constant throughout the experiment and any selection of litter types by the salamander was believed due to the nature of the litter.

At the termination of the experiment, the litter was removed, always starting with the middle section, and placed in large plastic boxes. The location of each animal was noted as was its position within a litter type, i.e., in bark crevices or on the paper towels under the bark. Animals not found under cover, or found between two litter types, or on the wall of the box were eliminated from the tally.

Preliminary Studies

Experiments were undertaken at room temperature (22^{o}C) for 683 hours. A mean time per habitat selection trial was 15 hours, 18 minutes with a variety of combinations of size classes, i.e., adults, immature and young; all young; all adults, or all immature being examined. The most common combination used was nine animals: three adults, three immature, and three young.

During an experimental run each animal was introduced three times for a test of selection preference while a constant light source provided illumination of approximately 18 foot-candles.

Temperature was measured by a YSI tele-thermometer (Model 43 TD). The probe lead extended into the box through a tight hole, which positioned the probe to rest on the paper towel surface where the temperature range was maintained at $22^{\circ}C \pm 1^{\circ}C$.

Eight-Hour Studies at Various Temperatures

For this series of experiments the fiberglass habitat box was housed on a foam rubber base in a Percival plant growth lab (Model E-57) with constant illumination of approximately 17 foot-candles.

Temperature readings were taken as in the preliminary studies, but inside the growth lab with the door closed, where experimental temperatures of 10, 20, and $25^{\circ}C \pm 1^{\circ}C$, were maintained.

The 24-hour day was divided into three eight hour trial periods (7 a.m. - 3 p.m., 3 p.m. - 11 p.m., and 11 p.m. - 7 a.m.) at which times six to eleven animals were released and allowed to select a litter type during a given eight hour period. At the conclusion of a trial each animal was collected, placed in a polystyrene box, and housed in a darkened incubator at $15^{\circ}C$, while another trial group of animals was released in the habitat box. Each animal was thus alternately tested eight hours and rested eight hours over a 48 hour period.

A chi-square analysis was applied to aid in interpretation of the results of these tests. When a chi-square analysis revealed a significant deviation from the 1:1:1 ratio expected by chance it was considered an indication that the type of litter influenced habitat selection.

Respiration Studies

During the summer of 1968 and the spring and summer of 1969, respiration studies were undertaken to measure respiratory rates (oxygen consumption) of three size classes of animals: adults, immatures, and young (Table 2). The effects of three different temperatures, 10, 20, and 25°C, were measured as were the outcomes resulting from feeding and nonfeeding of the trial animals. The temperatures were measured within $\pm$ 0.5°C and respiratory measurements were frequently made more than once on the same animal.

All oxygen consumption measurements were by means of a Gilson differential respirometer (Model GRP 14). Trial animals were acclimated in a darkened incubator, at 15°C for at least seven days prior to an experimental run. Fourteen animals were utilized per run, with seven animals being fed vestigial-winged *Drosophila* and seven not fed for at least seven days prior to an experiment.

Each trial animal was removed from the acclimation temperature and placed in a 15 ml GME-130 reaction vessel of known weight. The vessel and animal were then weighed on a type H16 Mettler balance and the animal's weight was determined by subtracting the reaction vessel weight.

For the absorption of CO_2 respired by the animals, a 0.2 ml solution of 10% KOH (Umbreit, Burris and Stauffer, 1964) was added to the side sac of the reaction vessel and a small plastic screen was positioned in such a way as to protect the animal from the caustic KOH solution. A standard taper 7/15 venting plug was then used to close off the side sac.

Each of the 14 reaction vessels, with animals, was attached to the Gilson differential respirometer along with a reference flask containing 10 ml of distilled water.

Following an acclimation period of about one-half hour, an experimental run was made at either 10, 20, or 25°C. An experimental sample always consisted of one animal per reaction vessel. A respirometer shaking frequency of 91 oscillations per minute was maintained for a mean duration of 2.67 hours per experimental run.

Micrometer readings of the amount of oxygen consumed were given digitally in microliters (μl), and recorded every 15 to 30 minutes. Following a series of readings the operating valves of the respirometer were opened and the micrometers adjusted to a reference point. The respirometer's operating valves were then closed for the duration of the measurement.

Respiratory rates (Rr) were calculated by summing the µl of oxygen consumed per hour for each of the 14 animals; then the weight (wet weight), in grams, was divided into this figure. A Rr per gram (wet weight) per hour (Rr/g/hr) was thus attained.

Finally, for each group (fed and nonfed) the mean and standard deviation was calculated. All calculations were performed by the Oregon State University Control Data Corporation (Model 3300) Computer.

It should be mentioned that in this study the effect of rhythms was not taken into account, except that approximately one-half of the measurements were made before noon and one-half in the afternoon (Norris, Grandy and Davis, 1963).

Population Characteristics

Throughout the course of this study, population composition was described by analysis of the following characteristics: (1) dorsal and ventral color patterns, (2) melanophore and leucophore or iridophore development, (3) snout-vent, tail length measurements, (4) reproductive state of males and females, (5) reproductive cycle and growth patterns under laboratory conditions, and (6) survivorship records. These data were used to infer the state of reproductive readiness and stage of maturation of natural populations.

Color Patterns

All observations of color were made on living animals with an AO cycloptic microscope with 10X eyepieces.

The dorsal surface was described by dividing the snout (portion anterior to a transverse line across the front of the eyes) into three categories based upon the percent of iridophore invasion:

Category I $<$ 25%
Category II 25% - 75%
Category III $>$ 75%

Five other dorsal body locations were described: (1) the paratoid gland, (2) the junction of the fore-limb and body, (3) the mid-point of the trunk, (4) the junction of the rear legs and the body, and (5) the mid-dorsal line of the tail (Figure 2A).

An analysis of each body location directed attention to the colors present. Brassy, coppery (reddish), golden, and blue were chosen as close representations of the color schemes perceived. These colors were then utilized in making sketches, upon outlines, of the dorsal surface of each animal examined. While the terms used to describe color were developed from personal impressions, a more exact description based on the work of Maerz and Paul (1930) is included for a detailed reference: brassy (Pl. 10L 12), coppery (Pl. 1G 12), golden (Pl. 11L 7), and blue (Pl. 33K 1).

Throughout this investigation the color and location of leucophores

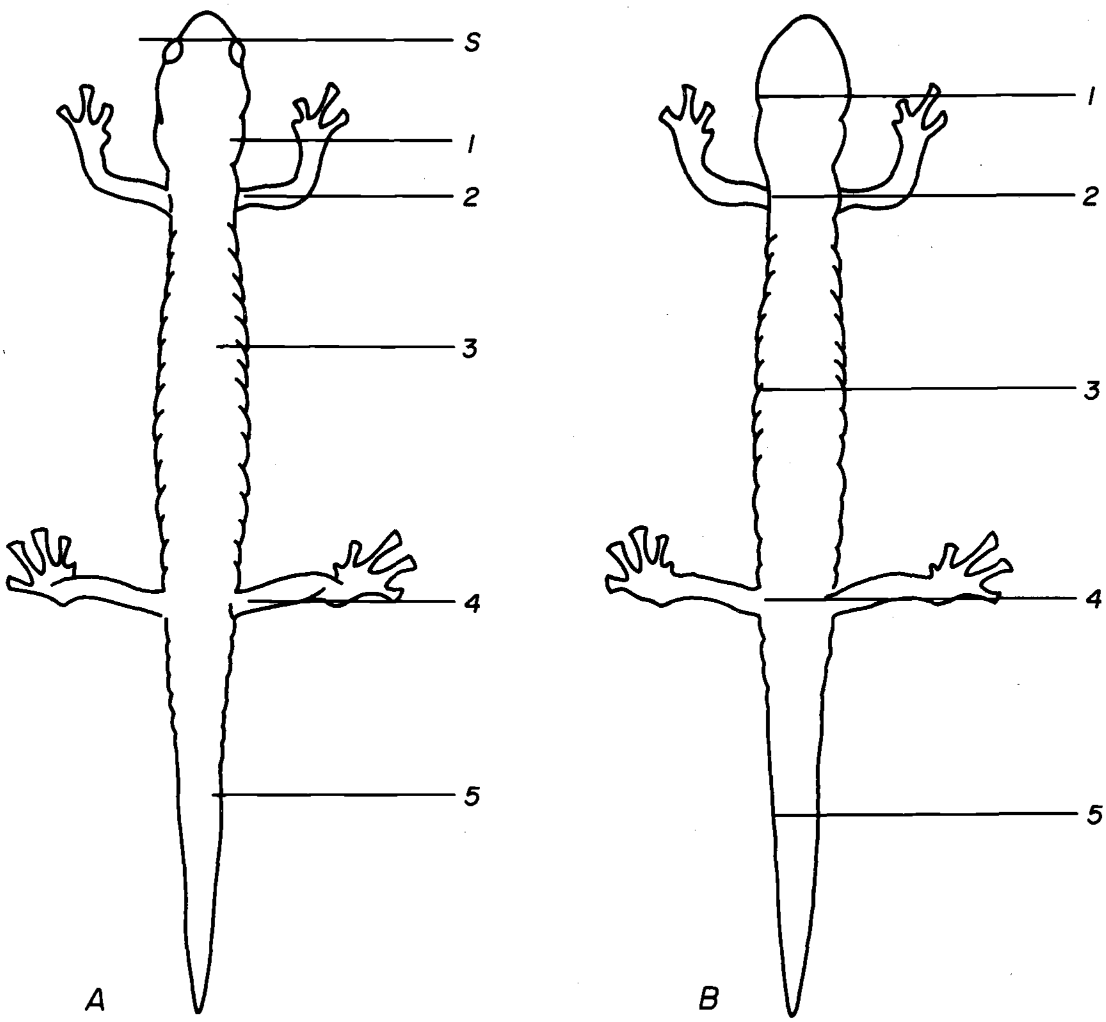


Figure 2. A. The dorsal location of the descriptive site of iridophore invasion on the snout (S) and the locations of the color pattern descriptions (1-5).
B. The ventral location of leucophore count sites (1-5).

was noted in relation to the size class and age of individuals.

To substantiate and further the descriptions of color, the ventral surface was divided into approximately the same five transverse sections as was the dorsal surface, for a description of the melanophore network, with the following code established:

- M I - Tight continuous network of melanophores
- M II - Melanophore network broken, especially on the throat and chin
- M III - Close-set but separate stellate melanophores
- M IV - Close-set, rounded melanophores

A count of leucophores was made at each transverse section (Figure 2B) and a count pattern established for each animal.

An attempt was made to relate color patterns and melanophore development to the type of habitat where the animals were collected.

No chemical analyses of pigments were made and the terms applied to chromatophores are used conditionally throughout this study (Hendrickson, 1954).

Reproductive Studies

The adult and immature animals were sexed by analysis of the inner vent and a series of 115 animals was killed in chloretone, placed in 10% formalin for 24 hours, washed in water, and preserved in 65% ethyl alcohol (Sayler, 1966). The forms were utilized as references for the dissections which followed.

Micrometer caliper measurements of body lengths were taken as snout-vent (tip of snout to posterior angle of vent) and tail length (posterior angle of vent to the tip of the tail). All measurements were to the nearest 0.5 mm. Since many of the individuals examined had incomplete tails, the snout-vent (S-V) measure was adopted as the basic measurement for comparative purposes. About 260 preserved and fresh animals were examined to determine the reproductive state of males and females.

Dissections were performed by making two incisions through the skin and underlying muscle layer on each side of the vent, parallel to the cloacal aperture, and extending as a single incision anteriorly to the front legs. Transverse cuts at the anterior and posterior end of the longitudinal incision allowed the skin and muscle to be pinned down. The organs covering the reproductive tract were removed, thus exposing the desired locations.

The males were analyzed for length, width and pigmentation of the testes. An attempt was made to locate areas of active spermatogenesis by observation of the shape of the testes, i.e., when the germ cells degenerated (following the spermatogenic wave), the size of the testis was reduced at this point (Sayler, 1966). A section of the testis was removed, crushed on a glass slide, and observed microscopically for the presence of sperm. The vasa deferentia were analyzed for pigmentation, presence of sperm, and the diameter measured at three points (anterior, mid, and posterior).

The females were examined for presence of eggs with the number in the right and left ovary noted, as was the diameter, stage of development, and color. The length and width of the oviduct and the diameter of the ostium were also measured. The spermatheca was exposed and its size and color described. The presence of sperm was noted by crushing the removed spermatheca on a glass slide and examining it with a microscope. The cloaca was analyzed for the presence of spermatophore remnants.

To more conclusively describe the life cycle of *Aneides ferreus*, an attempt was made to observe the following aspects under laboratory conditions: (1) spermatophore deposition, (2) sperm cap transfer to the female, (3) deposition of eggs, (4) hatching of young, and (5) the time of initial feeding by the young.

Survivorship Studies

Survivorship records of animals maintained in the laboratory were kept throughout the study. An analysis of the number of deaths of adults, immature, and young classes of animals was attempted.

RESULTS

Habitat Preference Studies

The influence of litter type upon the selective preference of experimental animals, under trial conditions, is summarized in Table 3 from data found in the Appendix (Tables 2-6). These data clearly show that there was a significant selection of litter type by members of each natural habitat group (Table 1) in all trials and at every temperature except in the trial utilizing the smallest (N = 45) number of individuals, at 25°C.

Preliminary Studies

The results of the preliminary studies conducted at room temperature (22°C) and utilizing only the animals collected from an urban habitat of boards, rock, and wet rug (Table 1), are illustrated in Table 4 from data found in the Appendix (Table 1).

There was a significant difference in the pattern of selection of habitat types in all cases. It was apparent that bark litter was selected as a preference (62.2%) over leaves (19.5%) and rock (18.3%) as shown in Table 4. When these data were analyzed by size classes, the trend discussed above continued, with bark litter being preferred by adults (73.3%), immatures (66.7%), and young (55.4%) (Figure 3).

Eight-Hour Studies at Various Temperatures

To further determine the effect of litter types upon the selective

Table 3. Chi-square analysis of the experimental results in habitat selection by <u>Aneides ferreus</u>. N = the total number actually counted in trials at different temperatures (10, 20, and 25°C).

Number (N =)	Natural habitat	Trial temperature (°C)	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
			Group I				
302	Boards,	10	Rock	109	115.3	36.0	
	rock,		Bark	184	115.3	61.0	
	wet rug		Leaves	9	115.3	3.0	153.1^{**}
254	Boards,	20	Rock	85	84.7	33.4	
	rock,		Bark	162	84.7	63.8	
	wet rug		Leaves	7	84.7	2.8	141.9^{**}
229	Boards,	25	Rock	60	76.3	26.2	
	rock,		Bark	161	76.3	70.0	
	wet rug		Leaves	8	76.3	3.5	158.6^{**}
			Group II - A and B combined				
352	Bark,	10	Rock	141	117.3	40.1	
	boards,		Bark	132	117.3	37.5	
	rocks		Leaves	79	117.3	22.4	19.1^{**}
351	Bark,	20	Rock	138	117.0	39.3	
	boards,		Bark	146	117.0	41.6	
	rock		Leaves	67	117.0	19.1	32.3^{**}
388	Bark,	25	Rock	165	129.3	42.5	
	boards,		Bark	178	129.3	45.9	
	rock		Leaves	45	129.3	11.6	83.2^{**}

Table 3. (Continued).

Number (N =)	Natural habitat	Trial temperature (°C)	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
			Group II A				
144	Rocks	10	Rock	58	48.0	40.3	
			Bark	58	48.0	40.3	
			Leaves	28	48.0	19.4	12.5**
147	Rocks	20	Rocks	65	49.0	44.2	
			Bark	51	49.0	34.7	
			Leaves	31	49.0	21.1	11.9**
124	Rocks	25	Rocks	62	41.3	50.0	
			Bark	49	41.3	40.0	
			Leaves	13	41.3	10.0	31.1**
			Group II B				
208	Bark and boards	10	Rock	83	69.3	40.0	
			Bark	74	69.3	35.5	
			Leaves	51	69.3	24.5	7.8*
204	Bark and boards	20	Rock	73	68.0	35.8	
			Bark	95	68.0	46.5	
			Leaves	36	68.0	17.7	26.1**
205	Bark and boards	25	Rock	84	68.3	41.0	
			Bark	101	68.3	49.0	
			Leaves	20	68.3	10.0	53.3**

Table 3. (Continued).

Number (N =)	Natural habitat	Trial temperature (°C)	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
			Group III				
111	Bark, rock	10	Rock	47	37.0	42.3	
			Bark	56	37.0	50.5	
			Leaves	8	37.0	7.2	35.2**
82	Bark, rock	20	Rock	37	27.3	45.1	
			Bark	42	27.3	51.2	
			Leaves	3	27.3	3.7	32.95**
45	Bark, rock	25	Rock	18	15.0	40.0	
			Bark	18	15.0	40.0	
			Leaves	9	15.0	20.0	3.60

**Significant at 1% level.

*Significant at 5% level.

Table 4. Chi-square analysis of the experimental results in habitat selection by combined adult, immature, and young *Aneides ferreus* at room temperature (22°C). Individuals collected in habitats of boards, rock, and wet rug. N = 431.

Location	Observed frequency	Theoretical frequency	Percent of N
Rock	79	143.6	18.3
Bark	268	143.6	62.2
Leaves	84	143.6	19.5
Chi-square value - 161.5**			

**Significant at 1% level.

Table 5. A summary of the group designations used in habitat selection studies.

Experimental Group		Habitat
I		Boards, rock, and wet rug
II	A	Rock
	B	Bark and boards
III		Mixed bark and rock

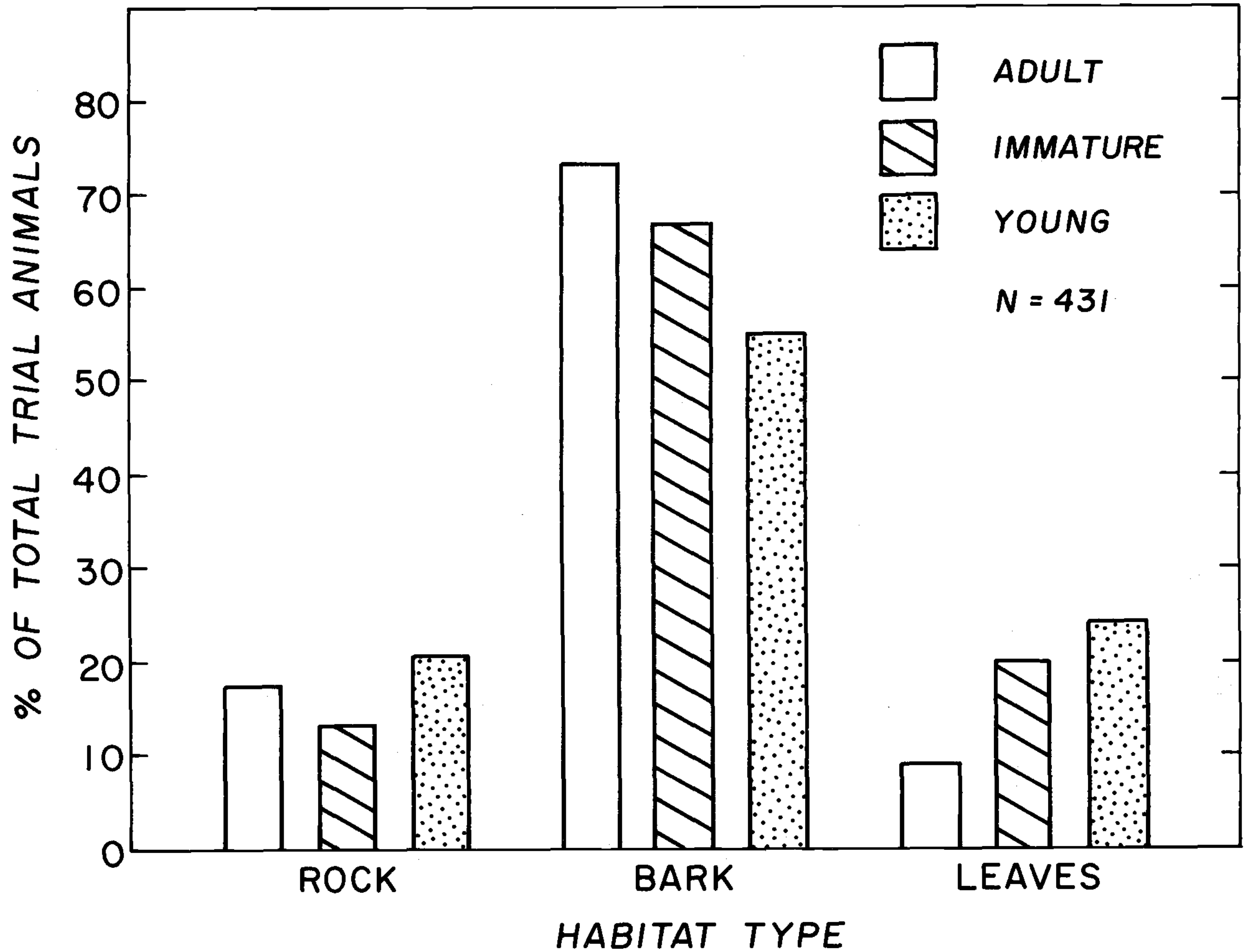


Figure 3. Each column represents the percent of Aneides ferreus of experimental Group I (collected in an urban habitat of boards, rock, and wet rug) selecting specific habitat types at an experimental trial temperature of 22°C.

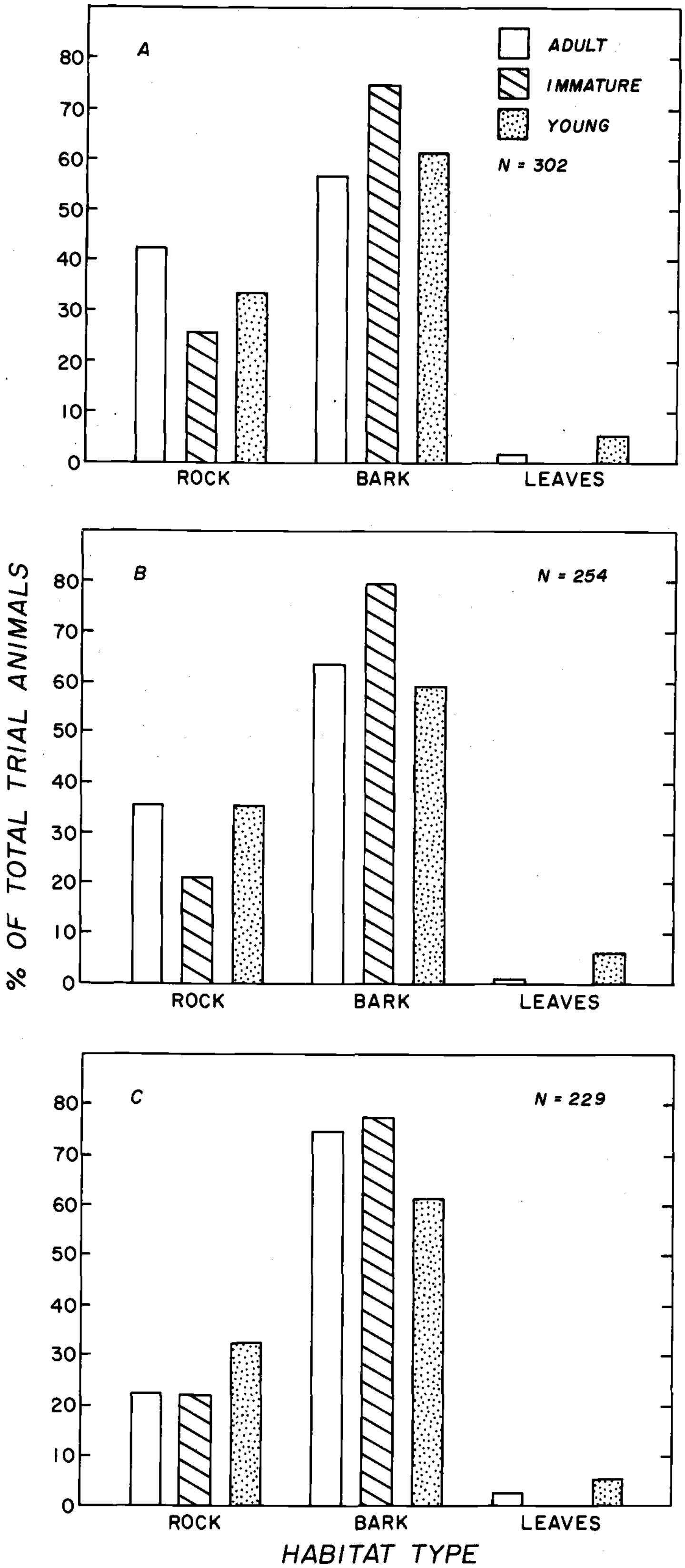
preference of habitats, eight-hour trials, conducted at 10, 20, and 25°C, were analyzed in relation to the native habitat of the animals, as shown in Appendix Tables 2-6.

Table 5 is a summary of groups used in the experiments (explained on pages 9 and 11). Groups II A and II B were tested both separately and together.

Table 3 illustrates the results of the trials with Group I animals, indicating significant choice of bark litter at all three temperatures, i.e., 61% (10°C), 63.8% (20°C), 70% (25°C). Further analysis of the data (Table 3) shows a less clear choice of bark litter by members of experimental Group II (both A and B); in fact, at 10°C, rock litter was selected with a slight preference to bark litter (40.1% to 37.5%, respectively). At 20 and 25°C there was only a small preference shown for bark litter. Individuals in Group III displayed a more clear-cut selective pattern for bark litter at 10 and 20°C but an equal (40%) selection of bark litter and rock litter at 25°C.

To better understand the relationships between the selective preference of each size class (adult, immature and young) and the type of litter selected by each at experimental temperatures of 10, 20, and 25°C, the following observations were made. Adults in Group I were less likely to select rock and more likely to select bark as the temperature increased from 10 to 25°C (Figure 4 A-C). An opposite

Figure 4 A-C. Each column represents the percent of *Aneides ferreus* of experimental Group I (collected in an urban habitat of boards, rock, and wet rug) selecting specific habitat types at three trial temperatures: A. 10°C, B. 20°C, and C. 25°C.



A
ADULT
IMMATURE
YOUNG
N = 302
B
N = 254
C
N = 229
% OF TOTAL TRIAL ANIMALS
0
10
20
30
40
50
60
70
80
ROCK
BARK
LEAVES
HABITAT TYPE

trend was demonstrated in Group II animals, from boards, bark, and rock habitats (Figure 5 A-C), where the adults' frequency of selecting rock litter increased as the temperature increased from 10 to 25°C. The adults (Group II A or II B) that were collected in either rock or bark and board habitat also displayed a slight increase in the frequency of selecting rock litter with an increase in temperature (Figures 6 A-7 C).

Adult *Aneides ferreus* in Group III exhibited a similar trend, with a peak for selection of rock litter (60%) occurring at 20°C (Figure 8 A-C). The choice of bark litter decreased with an increase of temperature in adults of this group.

Figure 4 A-C shows the selection of leaf litter by adult Group I animals to be less than 3% with no definite trend established, while individuals in Group II, whether collected from rock, bark, and boards; rock only; or bark and boards only, selected leaf litter less frequently with increased temperature (Figures 5 A - 7 C). Adults in Group III displayed no definite trend in their selective pattern of leaf litter at different temperatures (Figure 8 A-C).

Figure 4 A-C shows that the immature animals in Group I displayed a high preference for bark litter at each of the three trial temperatures: 74.3% (10°C), 78.8% (20°C), and 77.8% (25°C). During these trials, the preference for rock litter remained rather stable: 25.7% (10°C), 21.2% (20°C), and 22.2% (25°C). It is interesting

Figure 5 A-C. Each column represents the percent of *Aneides ferreus* of experimental Group II (collected in a habitat of bark, boards, and rock) selecting specific habitat types at three trial temperatures: A. 10°C, B. 20°C, and C. 25°C.

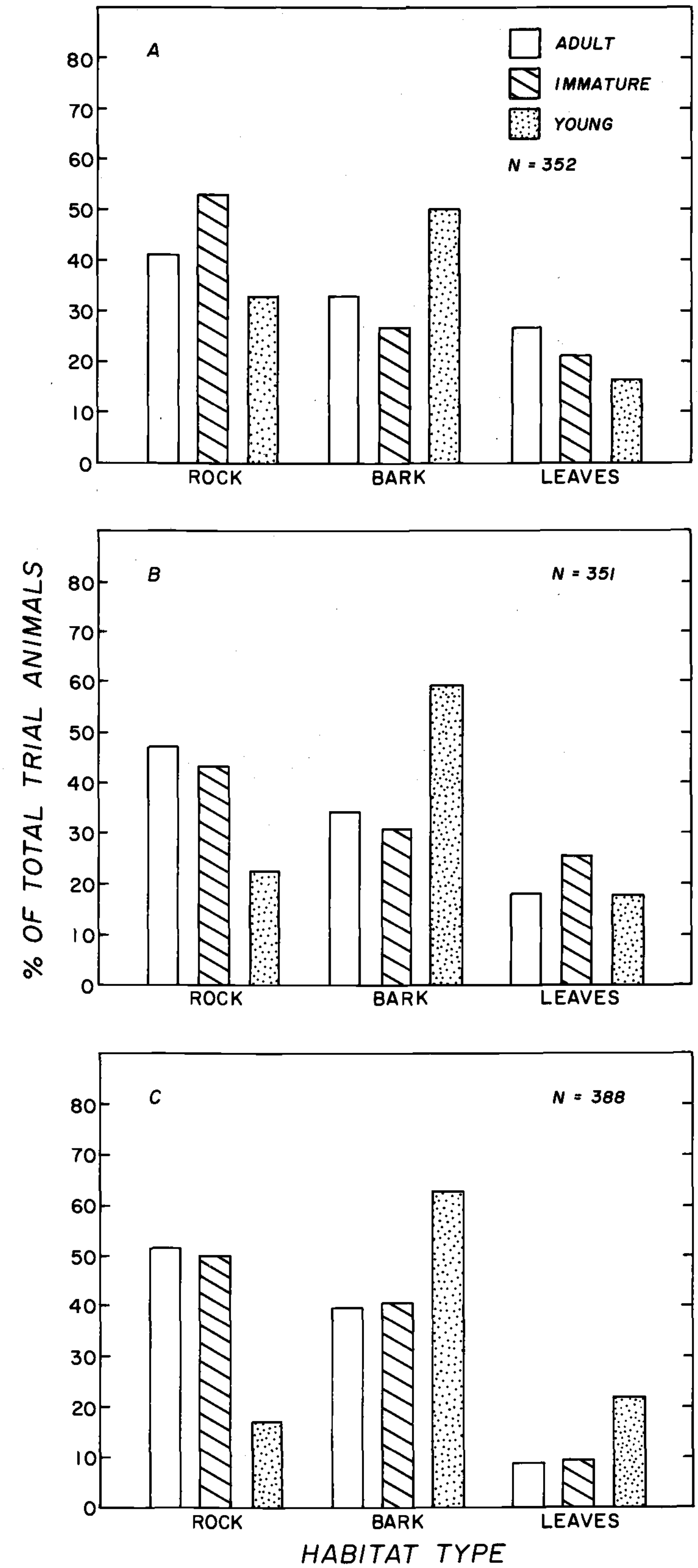

A
ADULT
IMMATURE
YOUNG
N = 352
B
N = 351
C
N = 388
% OF TOTAL TRIAL ANIMALS
0
10
20
30
40
50
60
70
80
ROCK
BARK
LEAVES
HABITAT TYPE

Figure 6 A-C. Each column represents the percent of *Aneides ferreus* of experimental Group II A (collected in rock habitats) selecting specific habitat types at three trial temperatures: A. $10^{o}C$, B. $20^{o}C$, and C. $25^{o}C$. These individuals were combined with bark and board animals as illustrated in Figure 5 A-C.

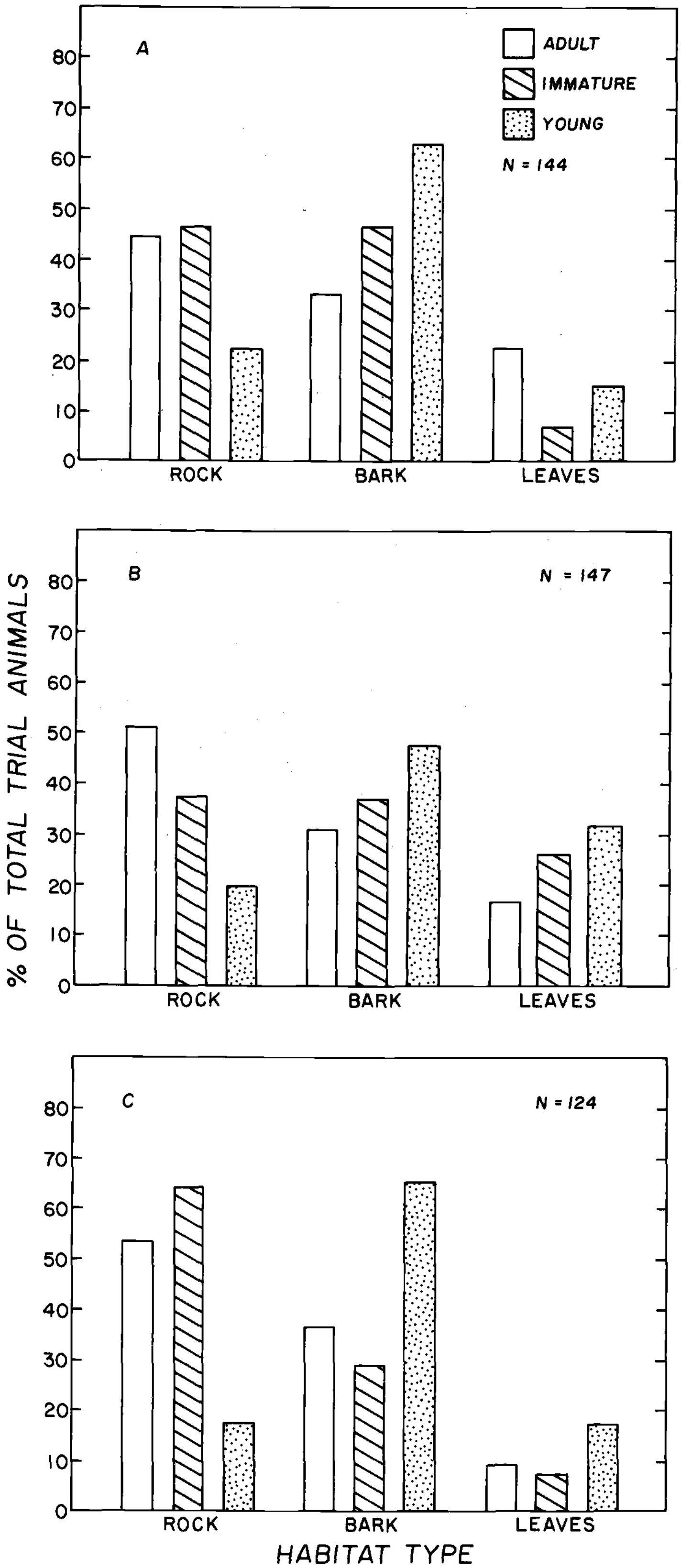

A
ADULT
IMMATURE
YOUNG
N = 144
B
N = 147
C
N = 124
% OF TOTAL TRIAL ANIMALS
0
10
20
30
40
50
60
70
80
ROCK
BARK
LEAVES
HABITAT TYPE

Figure 7 A-C. Each column represents the percent of *Aneides ferreus* of experimental Group II B (collected in bark and board habitats) selecting specific habitats at three trial temperatures: A. 10°C, B. 20°C, and C. 25°C. These individuals were combined with rock animals as illustrated in Figure 5 A-C.

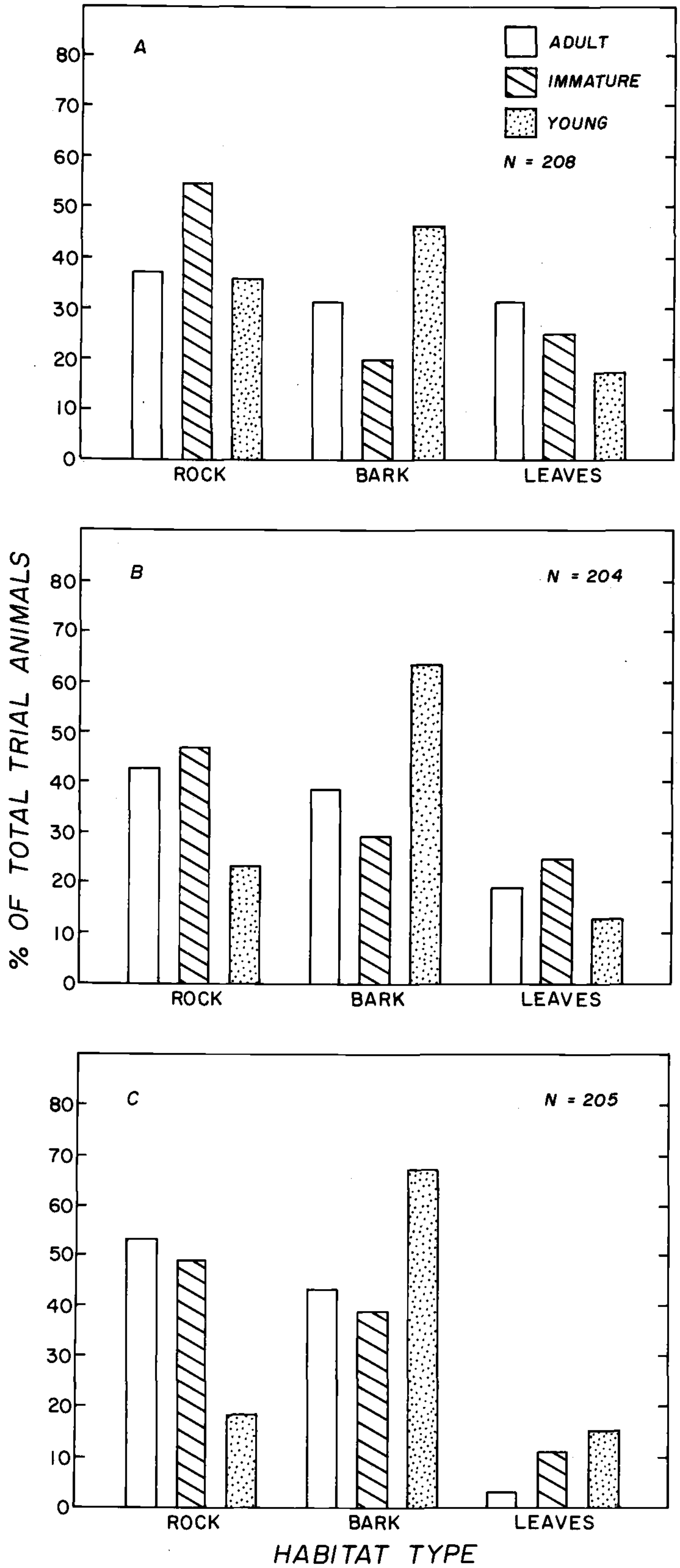
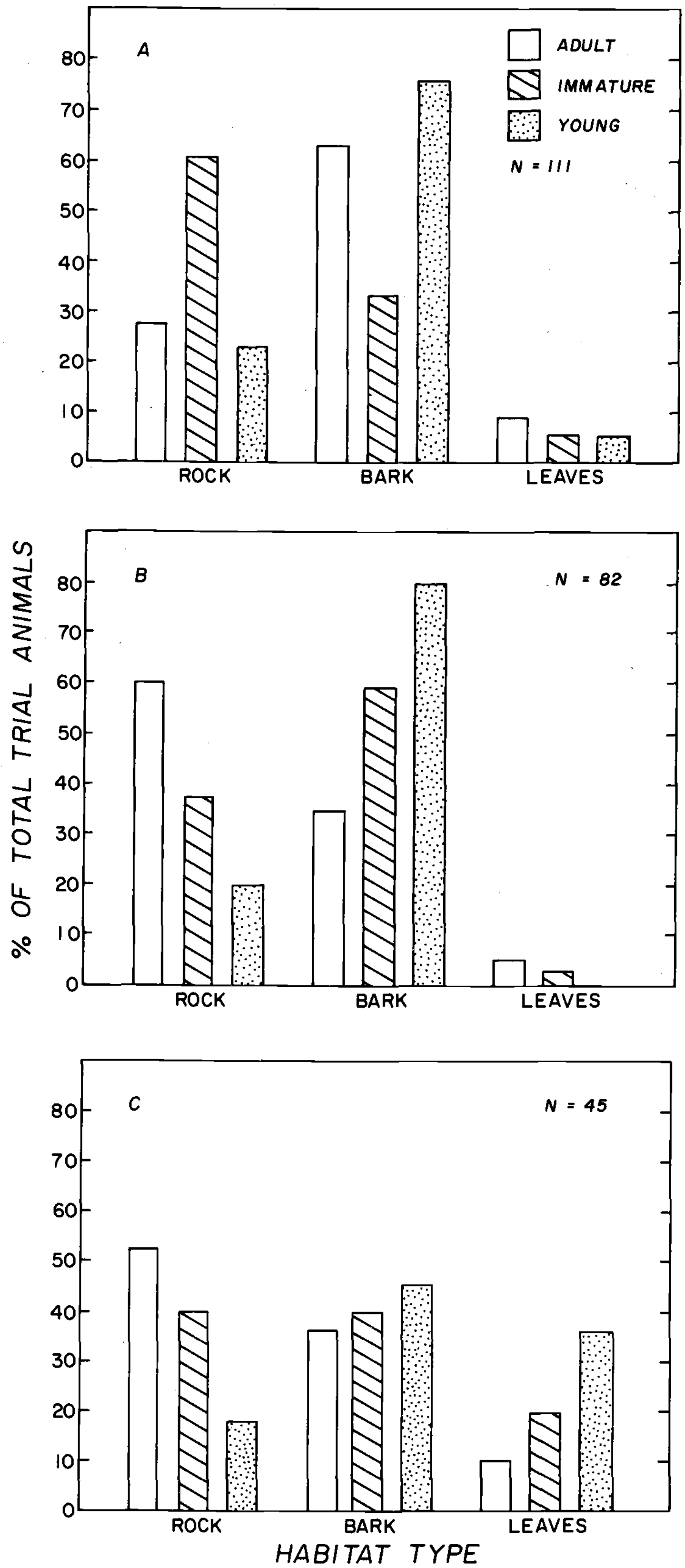

A
ADULT
IMMATURE
YOUNG
N = 208
0
10
20
30
40
50
60
70
80
ROCK
BARK
LEAVES
B
N = 204
% OF TOTAL TRIAL ANIMALS
C
N = 205
HABITAT TYPE

Figure 8 A-C. Each column represents the percent of <u>Aneides ferreus</u> of experimental Group III (collected in a mixed habitat of bark and rock) selecting specific habitats at three trial temperatures: A. $10^{\circ}C$, B. $20^{\circ}C$, and C. $25^{\circ}C$.


A
ADULT
IMMATURE
YOUNG
N = 111
B
N = 82
C
N = 45
% OF TOTAL TRIAL ANIMALS
ROCK
BARK
LEAVES
HABITAT TYPE

to note that leaf litter was not selected by this experimental group.

Immature forms in Group II selected rock litter as a preference at each of the three trial temperatures: 52.8% (10°C), 43.6% (20°C), and 50% (25°C), respectively (Figure 5 A-C). Figure 6 A-C indicates no difference in choice between rock or bark litter at 10 or 20°C but a clear choice of rock litter over bark litter at 25°C by the individuals collected from rock habitats (Group II A).

Figure 7 A-C indicates that immature individuals of Group II B, collected in bark, selected rock litter as a preference at each of the three trial temperatures, but with a less preferential choice at higher temperatures (20 and 25°C). Group II animals, regardless of native habitat, displayed no definite trend in regard to selection of leaf litter, except that at 20°C the leaf litter was selected at an equal or greater percentage of times than at 10 or 25°C.

An analysis of Figure 8 A-C shows that the immature animals in Group III departed from a high selection of rock litter (60.8%) at 10°C to a selection of bark litter, to a greater extent, at 20°C. At 25°C, rock litter and bark litter were selected with the same frequency.

The activity of this group in selecting leaf litter would seem to indicate that the leveling off of choices between bark litter and rock litter at 25°C (Figure 8 C) resulted in an increase in the selection of the leaf litter.

A definite pattern was shown by the young size class in the selective preference of bark litter in every trial and at each experimental temperature (Figures 4 A - 8 C). A more variable pattern of selective preference was demonstrated by the young in the order of choice between rock litter and leaf litter, with a general preference for rock litter. The only exceptions to this pattern were at higher temperatures (20 or 25^{o}C), when leaf litter was selected by young with an equal or greater frequency than was rock litter (Figures 5 C, 6 B, 6 C, and 8 C).

Respiration Studies

The results of experiments designed to measure the respiratory rates of three size classes of animals under the effect of different temperatures (10, 20, and 25^{o}C) and feeding regime are summarized in Table 6 and shown graphically in Figure 9.

At 10^{o}C the oxygen consumption was low with no significant difference between the adults and immature or between fed and nonfed of these size classes. However, the young size class displayed a significantly higher mean respiratory rate at 10^{o}C than either immature or adults.

At 20^{o}C the adult size class consumed oxygen, whether fed or nonfed, at the same rate, 57.23 and 57.99, respectively. The immature group displayed a marked increase in respiration, especially the fed animals (96.15), at this temperature. The young

Table 6. Mean respiratory rates for Aneides ferreus based upon the μl of oxygen consumed per gram (wet weight) per hour (μl/g/hr).

Animal size (range in g)	10°C		20°C		25°C	
	Fed	Nonfed	Fed	Nonfed	Fed	Nonfed
Adult (2.01 - 5.40)	31/31.75 ± 9.5*	35/29.94 ± 9.6	40/57.23 ± 13.4	39/57.99 ± 11.8	37/77.63 ± 15.4	36/75.15 ± 12.5
Immature (0.44 - 2.00)	20/29.93 ± 11.1	19/28.77 ± 8.9	16/96.15 ± 41.9	17/73.46 ± 24.7	33/99.29 ± 30.3	32/85.16 ± 20.7
Young (0.09 - 0.43)	17/52.58 ± 21.8	18/57.80 ± 24.3	20/116.22 ± 35.6	17/65.58 ± 33.3	19/161.21 ± 54.8	21/114.10 ± 37.8

*The number of measurements precedes the slant. Standard deviations are shown.

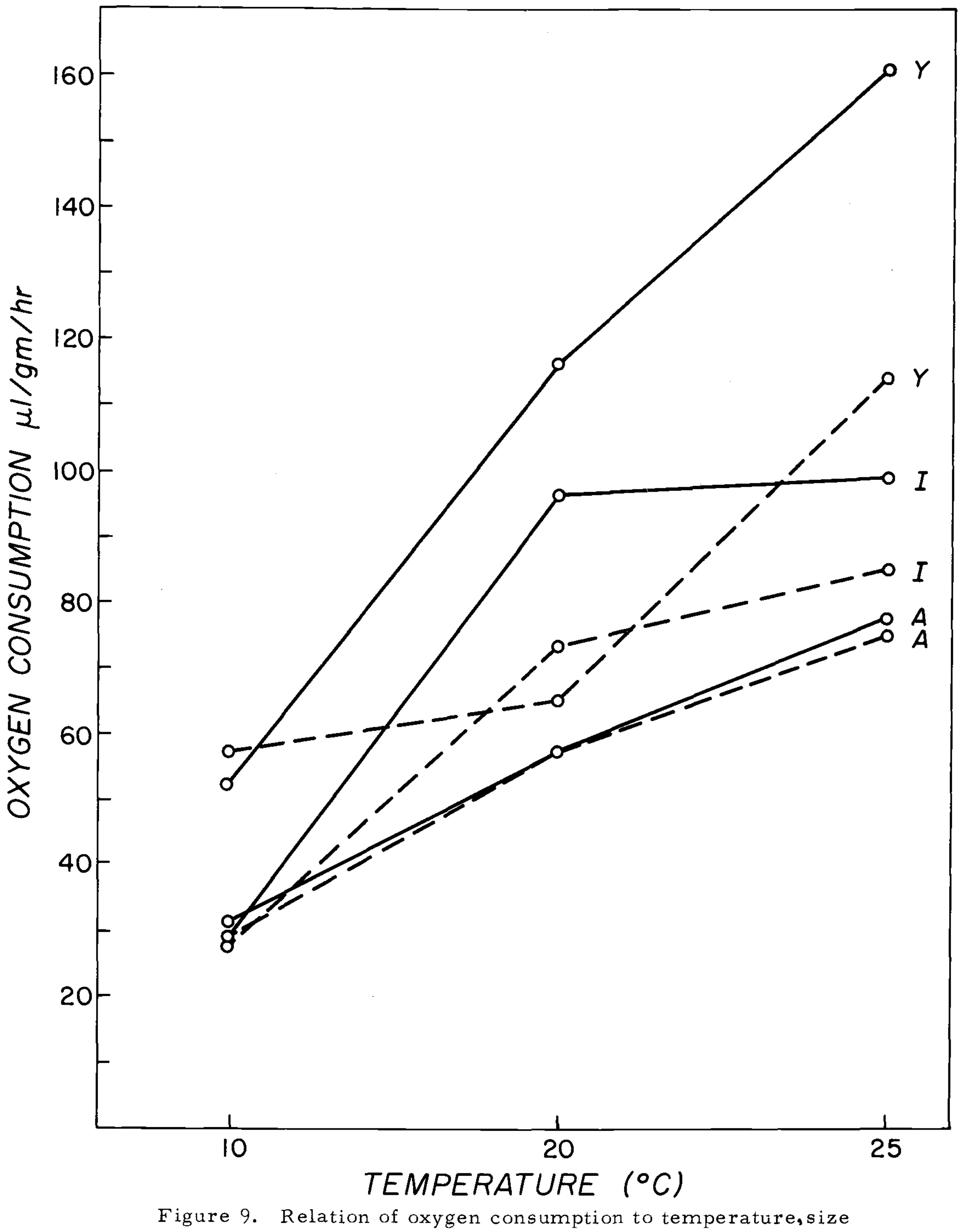


Figure 9. Relation of oxygen consumption to temperature, size class, and feeding regime of Aneides ferreus. Y = Young; I = Immature; A = Adult. Complete lines (—) are fed, and broken lines (--) are nonfed.

nonfed individuals, although increasing their respiration rate from 57.80 at 10°C to 65.59 at 20°C, displayed much less increase than the immature at the same temperature. Figure 9 shows the point where the nonfed immature animals actually had a higher oxygen consumption (73.46) than did the nonfed young (65.59). The young fed individuals showed the highest respiratory rate (116.22).

At 25°C the adults, as at 20°C, displayed no significant differences in oxygen consumption between the fed and nonfed members (77.63 and 75.15, respectively). There was, however, a steady increase in respiration when compared to the oxygen consumed at 20°C (Figure 9).

The immature size class showed the most pronounced leveling off in oxygen consumption between 20 and 25°C, as evidenced in Figure 9. There was once again a significant difference in rate of respiration between the fed (99.29) and nonfed (85.16) individuals.

In animals of the young size class, the rate of oxygen consumption reached its highest level at 25°C. There was a marked difference in respiration between the fed and nonfed animals, with rates of 161.21 and 114.10, respectively.

Color Patterns

A detailed description of 255 living *Aneides ferreus* from all collecting locations (Table 1) resulted in categorization of the three size classes (adult, immature, and young) into groups based upon dorsal and ventral surface color patterns (Figure 2 A-B). In general terms the dorsal parts of the body were usually uniformly brownish with metallic or brassy flecking that tended to be concentrated on the snout, shoulders and mid portion of the tail. Larger, older animals generally lacked this flecking and appeared a uniform dark brown, while the young animals often appeared to have the flecking concentrated into a coppery or brassy dorsal stripe. The ventral surface ranged from nearly black to grayish or dusty.

With regard to dorsal surfaces of Aneides ferreus, Table 7 illustrates the complete description of color patterns in the three size classes of animals studied. Seventy-five percent of the adults were characterized by an invasion of iridophores of less than 25% (Category I) on the snout surface while about 90% of the body areas analyzed displayed golden specks.

Leucophores, in this size class, were mostly white (54%) or yellow (40.2%), with only 5.8% blue in color.

The immature individuals displayed a higher concentration of iridophores on the snout surface with 45.9% of these animals ranging between 25% - 75% coverage (Category II). Golden specks were the most common color of the body areas, as was true in the adults, but more dorsal areas (paratoid gland 30.9%, shoulder 30%, back 29.3%, leg 30%, and tail 49%) were characterized by a coppery (reddish) color.

Table 7. A summary of the dorsal color pattern descriptions of *Aneides ferreus*. The snout categories were based upon the percent of iridophore invasion (I < 25%, II 25%-75%, III > 75%). Each number represents the percentage presence of a color.

Subject described	Description	Size class		
		Adult	Immature	Young
Snout category	I	75.0	40.6	9.9
	II	23.8	45.9	42.2
	III	1.2	13.5	47.9
Snout color	Brassy	1.2	2.6	4.1
	Coppery	1.2	15.4	49.3
	Golden	97.6	82.0	46.6
Paratoid gland color	Brassy	1.1	2.4	5.6
	Coppery	4.6	30.9	76.4
	Golden	94.3	66.7	18.0
Shoulder color	Brassy	1.2	2.5	5.6
	Coppery	2.4	30.0	76.4
	Golden	96.4	67.5	18.0
Back color	Brassy	1.2	2.4	6.9
	Coppery	2.4	29.3	76.7
	Golden	96.4	68.3	16.4
Leg color	Brassy	1.2	2.5	1.4
	Coppery	1.2	30.0	69.9
	Golden	97.6	67.5	28.7
Tail color	Brassy	1.1	2.0	1.4
	Coppery	9.2	49.0	77.0
	Golden	89.7	49.0	21.6
Leucophore color	Blue	5.8	46.0	100.0
	White	54.0	48.0	-
	Yellow	40.2	6.0	-

About an equal percentage (46% and 48%) of the leucophores were of a blue or white color respectively, and only 6% were yellow.

Young animals showed a much greater invasion of iridophores on the snout than either the adult or immature group, with 47.9% of the type III Category (> 75% coverage) and 42.2% type II (25% - 75% coverage). Coppery was the most evident color of the body regions with 49.3% of the snout colors of this type, while golden snout color was exhibited in 46.6% of the young individuals. Colors tended to be concentrated into patches on the snout, paratoid gland, shoulders, legs, and tail.

Even higher percentages (over 75%) of coppery color were noted in the paratoid gland, shoulder, back, and tail of young animals, while the legs displayed 69.9% coppery color. Golden color, although common, was not as extensive as in the adult or immature groups. The analysis of leucophores in young Aneides resulted in the striking observation that 100% were blue.

The ventral melanophore network was described in 92 young, 59 immature, and 104 adult animals, with the following results noted: There was a definite trend toward a more broken melanophore network with an increase in size and age. By utilizing the classification code, discussed previously (p. 20) and illustrated in Figure 10, it was found that in young animals, 81.3% showed an M I pattern while 18.7% were of the M II category. To further illustrate this trend toward a

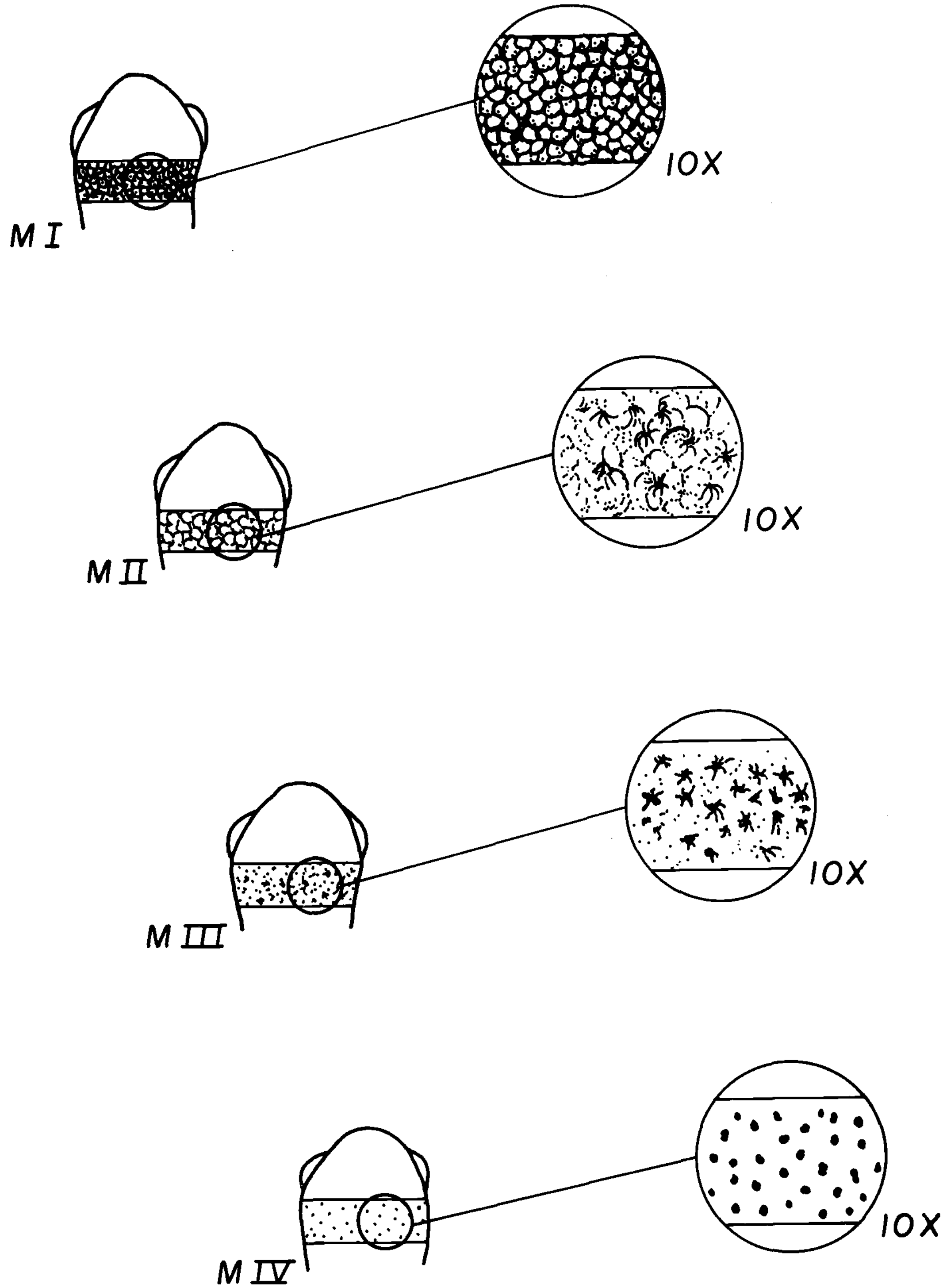


Figure 10. An illustration of the ventral melanophore network from the throat region of Aneides ferreus. M I - tight continuous network; M II - broken network (especially on throat and chin); M III - close-set but separate stellate pattern; M IV - close-set, punctate pattern.

more broken melanophore network in the immature size class, only 1.7% of the animals were of the M I pattern and 77.6% exhibited M II characteristics. This group also displayed M III pattern in 19% of the individuals and 1.7% had a melanophore development of the M IV type.

The adults showed only 0.9% M I pattern while 48.6% were of the M II type. The M III category was displayed by 42.9% of adults and 7.6% exhibited M IV characteristics.

To determine more completely the pattern of color pigments of the venter, a description of five transverse ventral sections per animal (Figure 2 B) resulted in an analysis of the number of leucophores present in 460 descriptive sections of young, 295 descriptive sections of immature, and 520 descriptive sections of adults.

Table 8 shows that with increased size and maturity (young to adult) there was a decrease in the number of leucophores present on the venter.

Table 8. A summary of the number of leucophores present in five transverse ventral sections of *Aneides ferreus* (Figure 2 B). Each mean value represents the frequency per descriptive section.

Size class	Mean $\pm$ SD	Range	Number of sections described
Young	3.0 ± 1.8	0-12	460
Immature	1.6 ± 1.5	0-7	295
Adults	0.82 ± 1.19	0-7	520

Five (four adults and one immature) aberrant individuals were analyzed in the course of studies of color descriptions. The following observations pertain to these individuals: All animals appeared an over-all light groundcolor with a few scattered melanophores concentrated in spots, flecked with gold. There was a concentration of orange color (probably due to blood circulation below the golden pigment) on the dorsal surface of the limbs and tail, not unlike the coloration of Ensatina eschscholtzi. The snout displayed iridophore invasion of Category I in four of the individuals and Category II in one animal. The dorsal leucophores were white and yellow.

The venter was characterized by wide-spread stellate melanophores that were larger and more dendritic than those of the typical Aneides ferreus of M IV Category (Figure 10).

Breeding and Early Life History

Prior to this study, observation of spermatophore deposition had not been reported for Aneides ferreus. On two occasions, during the course of this study, the results of this process were observed under laboratory conditions. The following is a report of those observations.

On May 6, 1969, a spermatophore (jelly stalk) was found attached to a wet paper towel covering the bottom of a polystyrene animal storage box which housed three adult male and one adult female clouded salamanders. The snout-vent measurements of the males were 60.0, 57.2, and 55 mm, while the female's was 62.7 mm.

The sperm cap had been detached from the jelly stalk and when the female was turned over and examined there was yellow-white matter extending from the vent. A microscopic examination showed this matter to be laden with living sperm. Further microscopic examination demonstrated a lack of sperm on the tip of the jelly stalk. On May 8 the female showed no remnant of a sperm cap extending from the vent; it was concluded that the female had completed transfer of the sperm cap into the vent.

The colorless jelly stalk of the spermatophore appeared to be shaped like a somewhat blunt dunce cap with the tip tilted from the vertical. This supporting jelly stalk measured 3.2 x 2.7 mm across the base and was 4.0 mm high. There were striations on the surface of the jelly and the base of the stalk flared outward to form a broad surface of attachment with the paper towel substrate, being similar in these respects to Plethodon glutinosus (Organ, 1960a).

On May 12, 1969, one male and the female discussed above were observed in close association, lying side by side. When touched lightly, the individuals demonstrated active movements during which time they became entwined by their tails with their bodies still parallel to each other. Upon examination of the wet paper towel floor covering, a second spermatophore (jelly stalk) was noted. It measured 2.8 x 2.8 mm across the base and was 3.1 mm high. The female once again displayed external signs of the sperm cap extruding from the vent and a slide preparation revealed active sperm. As in the first observation, no sperm were found at the tip of the jelly stalk.

Thus it would appear that the female in question had picked up two sperm caps in a period of five full days between the first spermatophore deposition (May 6) and the second deposition (May 12).

Eggs of Aneides ferreus have been previously found and reported by Dunn (1942) and Storm (1947, 1948), but in neither case was spermatophore transfer related to egg deposition. Four egg clusters were noted in the present investigation as follows: On June 27, 1968, eight eggs were discovered in a polystyrene container housing one adult female. One egg was resting alone on the wet paper towel floor and seven were attached to the wall in a series of 2, 1, 1, and 3 per cluster. The supportive gelatinous strands were twisted about and adhered to one another toward the point of attachment in the clusters of more than one egg, while the lone egg was supported by a single, non-twisted strand. By July 5, 1968 five of the eggs had disappeared, probably being eaten by the female; the female was removed at this time and the remaining three eggs molded and dried out by July 25. The eight eggs in this description measured approximately 5 mm in diameter and appeared to be a light cream color.

The second observation of egg deposition was noted on July 8, 1968, when a single egg, 5 mm in diameter, was laid at 10:30 a.m. and gone by 12:45 p.m. Cnce again it was assumed that the attending female had eaten the egg.

On July 24, 1968, five eggs (5 mm in diameter) were deposited in a row, with each attached individually to the wall of the polystyrene box. Fearing that the female would devour the eggs, as in the previous instances, she was removed, but all eggs were eventually lost due to mold.

The final and most successful observation began at 10:45 a.m. on July 28, 1968, when five eggs were found attached to the wall of a polystyrene box and one resting on the wet paper towel bottom. By 6:45 p.m., of the same day, four more eggs were attached to the wall of the container and the female was in close association with the eggs. It should be noted that the female did not feed during the entire course of incubation (approximately three months).

All eggs attached to the wall were suspended by supportive strands, twisted about each other. The eggs measured approximately 4.5 mm in diameter and were of the same color and appearance as those discussed previously (Figure 11).

On August 9, 1968, the single egg deposited on the paper towel was removed because of the development of mold and the female continued to guard those attached to the wall of the box. Another egg was removed because of mold on August 20, 1968.

Finally, on October 23, 1968, five eggs hatched with the young emerging by crawling head first up the wall of the container. The

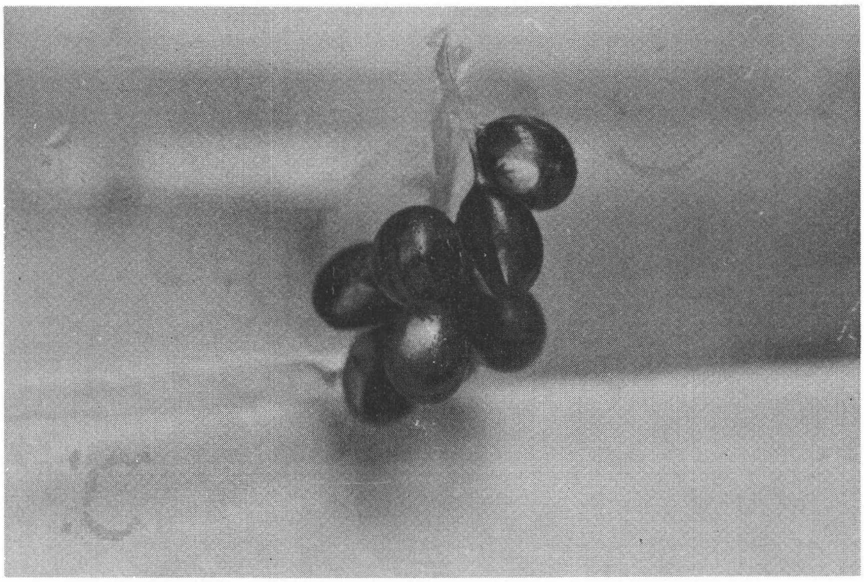


Figure 11. View showing a cluster of Aneides ferreus eggs deposited, under laboratory conditions, on July 28, 1968.

remaining three animals hatched one per day over the next three days (August 24, 25, and 26). All the newly hatched individuals were very active to the touch, displayed small, light gray gills; and had a mean total length of 25.9 mm (Table 9).

Table 9. The snout-vent, tail length measurements (in mm) of eight newly hatched _Aneides ferreus_. Mean total length = 25.9 mm.

Snout-vent length	Tail length
15.5	9.5
15.4	10.4
15.9	9.0
17.0	9.6
17.0	10.0
16.0	10.0
16.2	9.5
16.5	9.4

The first feeding was observed on November 5, 1968, when a vestigial-winged _Drosophila_ was stalked and eaten.

Reproductive Studies

Dissections were performed on preserved and freshly killed (chloretone) _Aneides ferreus_ collected during every month of the year except January. A number of animals analyzed were collected December 29, providing a good indication of January animals.

The reproductive organs of adult and subadult male and female animals were examined with the aid of a dissecting microscope. Due to the poor development of the reproductive organs in the subadult females the analysis proved to be incomplete in this group.

Sex of the animals was determined by the presence of cloacal papillae in males and cloacal folds in females. These differences are present in even the smallest individuals. Sexual maturity was estimated by means of the findings from dissections. Testes and vasa deferentia smears were utilized in determining the smallest snout-vent length at which sperm were present in the males (Tables 10 and 11).

The testes of the males ranged in color from gray to black and the vasa deferentia were always a dense black color. Most mature females possessed ovarian eggs; those termed the late stage of development were large (extremes of 2.6 to 3.6 mm) and yellowish as compared to the tiny, grayish-white eggs, termed the intermediate stage of development. These findings were very similar to those of Rossman (1959) in his study of Desmognathus in Florida and sourthern Georgia. Eggs termed early were very small and undeveloped (Table 12).

Reproductive Cycle of Males

The changes in the appearance of the testes and vasa deferentia in adult males during every month of the year except January are shown in Table 10. The testes of animals collected in all months were quite similar in size. Apparently there is no annual cycle involving change

Table 10. Condition of gonads of mature (adult) male *Aneides ferreus* in every month except January. The presence (+) or absence (-) of sperm is expressed in a percent of N (% of N). The $\bar{x}$ length of vasa deferentia is expressed in the number of times longer each is than the corresponding testis. All measurements are in (mm) and standard deviations are given.

Month	N	Length (S-V)	Testes					Vasa deferentia				
					Sperm				Sperm			$\bar{x}$ Length
			$\bar{x}$ length	$\bar{x}$ width	right	left	% of N	$\bar{x}$ diameter	right	left	% of N	length of testes
Jan	—											
Feb	3	53 ± 4.2	6.6 ± 1.0	1.2 ± 0.4	-	-	100	0.3 ± 0.13	+	+	100	3.1
Mar	10	55.9 ± 5.2	6.8 ± 1.8	1.9 ± 1.1	-	-	100	0.5 ± 0.72	+	+	87.5	2.7
									-	-	12.5	
Apr	11	53.8 ± 2.4	6.1 ± 1.7	1.3 ± 0.5	-	-	91	0.3 ± 0.19	+	+	100	2.7
					-	+	9					
May	7	49.4 ± 2.1	5.3 ± 1.08	1.5 ± 0.39	-	-	100	0.4 ± 0.21	+	+	100	2.3
Jun	4	60.2 ± 5.31	9.5 ± 2.35	3.0 ± 1.26	-	-	100	0.2 ± 0.11	+	+	25	2.2
									-	-	75	
Jul	8	60.9 ± 5.29	6.13 ± 1.07	1.8 ± 0.42	+	+	100	0.1 ± 0.04	+	+	100	2.5
Aug	2	60.1 ± 1.62	7.1 ± 1.56	2.0 ± 0.00	-	-	100	0.1 ± 0.15	+	+	50	2.5
									-	-	50	
Sept	2	59.0 ± 4.24	5.5 ± 0.57	1.3 ± 0.40	-	-	100	0.6 ± 0.10	-	-	100	2.2
Oct	1	63.0 ± 0.00	7.9 ± 0.00	2.0 ± 0.00	-	-	100	0.4 ± 0.00	+	+	100	2.5
Nov	2	55.0 ± 1.41	6.0 ± 1.15	1.2 ± 0.00	-	-	100	0.4 ± 0.21	-	-	50	2.7
									+	+	50	
Dec	10	61.8 ± 7.19	5.8 ± 1.77	1.8 ± 0.25	-	-	100	0.7 ± 0.16	+	+	100	3.2

Table 11. Condition of gonads of subadult male Aneides ferreus in every month except January, June, September, and December. The presence (+) or absence (-) of sperm is expressed in a percent of N (% of N). The $\bar{x}$ length of vasa deferentia is expressed in the number of times longer each is than the corresponding testis. All measurements are in (mm) and standard deviations are given.

Month	N	Length (S-V)	Testes					Vasa deferentia				
					Sperm				Sperm			$\bar{x}$ Length
			$\bar{x}$ length	$\bar{x}$ width	right	left	% of N	$\bar{x}$ diameter	right	left	% of N	length of testes
Jan	—											
Feb	2	38.0 ± 1.41	4.5 ± 0.57	1.0 ± 0.00	-	-	100	0.1 ± 0.04	+	+	50	1.5
									-	-	50	
Mar	8	44.9 ± 3.83	5.0 ± 1.10	1.0 ± 0.04	-	-	80	0.1 ± 0.08	+	+	63	3.0
					-	+	20		-	-	37	
Apr	7	48.4 ± 2.14	5.3 ± 1.06	0.8 ± 0.29	-	-	100	0.1 ± 0.04	+	+	100	2.2
May	7	45.5 ± 2.29	4.1 ± 0.32	1.0 ± 0.07	-	-	100	0.2 ± 0.12	+	+	100	2.1
Jun	—											
Jul	5	43.8 ± 1.30	4.6 ± 0.68	1.0 ± 0.47	-	-	100	0.2 ± 0.06	+	+	100	2.0
Aug	1	44 ± 0.00	5.5 ± 0.00	1.0 ± 0.00	-	-	100	0.1 ± 0.00	-	-	100	1.5
Sept	—											
Oct	1	46.0 ± 0.00	5.1 ± 0.00	1.3 ± 0.00	-	-	100	0.6 ± 0.00	-	-	100	2.5
Nov	1	47.0 ± 0.00	4.8 ± 0.00	1.0 ± 0.00	-	-	100	0.5 ± 0.00	-	-	100	3.0
Dec	—											

in over-all testicular size in adult males. However, it was possible to observe occasionally a slight swelling in a portion of the testes of mature individuals. This was assumed to be the location of active spermatogenesis, i. e., a point anterior to a slight constriction resulting from degeneration of cells following the spermatogenic wave (Sayler, 1966).

The presence of sperm in the vasa deferentia was noted in adult male animals collected in every month analyzed except September. Specific mature individuals displayed a lack of sperm in the vasa deferentia in March, June, August, and November (Table 10). Sperm were found in the vasa deferentia of subadult individuals collected in February, March, April, May, and July (Table 11). The length of the vasa deferentia was estimated by the number of times longer each was than the corresponding testis, i. e., right vasa deferentia, 2. 5 times as long as the right testis. These lengths ranged in the mature adult from 2. 2 to 3. 2 times the length of the testes (Table 10) and in subadults from 1. 5 to 3. 0 times the length of the testes (Table 11).

The diameter of the vasa deferentia ranged from 0. 1 to 0. 7 mm in mature adult males and displayed only slight variation throughout the year (Table 10). The subadult males displayed even less variation in size of the vasa deferentia (Table 11).

Subadult males (Table 11) were analyzed separately from the mature adults on the basis of their relatively smaller size and generally more incomplete development of the reproductive structures. However, the subadult males displayed less variation from the mature individuals than did the female subadults from the mature females (Tables 10-13).

The mature males' snout-vent measurements ranged from 49.4 to 63 mm in length, with a mean length of 57.1 mm. Subadult males' snout-vent measurements ranged from 38 to 48.4 mm in length, with a mean length of 44.7 mm.

The smallest male to possess sperm was a subadult collected in February with a snout-vent length of 38 mm.

Reproductive Cycle of Females

Tables 12 and 13 show the condition of the eggs and oviducts in the adult or the oviducts alone in the subadult females for every month except January. The mature females ranged from 50 to 65 mm in snout-vent length, with a mean snout-vent length of 58.1 mm. The subadult females' snout-vent measurements ranged from 23.2 to 47.5 mm in length with a mean length of 35.9 mm.

Dissection revealed the color composition of eggs to be generally orange and yellow (late stage of development) and white or creamy (intermediate and young stage of development) in adult females (Table 12).

No spermatophore remnants were found in any of the animals

Table 12. Condition of gonads of mature (adult) female Aneides ferreus in every month except January, September, October, and November. Individuals are placed in order by the stage of development of the eggs, i.e., L = Late; I = Intermediate; E = Early. The presence (+) or absence (-) of sperm es expressed in a percent of N (% of N). All measurements are in (mm) and standard deviations are given.

Month	N	Length (S-V)	Egg			Oviduct			Spermatheca		
			$\bar{x}$ No.	$\bar{x}$ diameter	stage of development	$\bar{x}$ length	$\bar{x}$ width	$\bar{x}$ diameter	size	sperm	% of N
Jan	–										
Feb	3	58.1 ± 3.81	11 ± 2.0	2.7 ± 0.45	L	38.8 ± 7.68	1.7 ± 0.60	7.6 ± 4.48	1.1 ± 0.11	+	100
	1	60.8 ± 0.00	6.5 ± 0.00	2.2 ± 0.00	I	49.0 ± 0.00	1.0 ± 0.00	8.6 ± 0.00	1.2 ± 0.00	+	100
	1	59.0 ± 0.00	41 ± 0.00	0.5 ± 0.00	E	30.0 ± 0.00	0.5 ± 0.00	3.0 ± 0.00	0.5 ± 0.00	-	100
Mar	6	57.7 ± 2.91	18 ± 4.24	2.6 ± 0.00	L	25.3 ± 0.95	2.2 ± 0.95	6.4 ± 0.72	1.1 ± 0.10	+	100
	2	57.5 ± 9.19	20 ± 12.72	1.8 ± 1.00	I	21.0 ± 5.65	0.9 ± 0.14	- - - -	1.0 ± 0.00	+	50
										-	50
	2	51.0 ± 0.00	50.0 ± 1.4	0.2 ± 0.95	E	20.5 ± 0.70	1.0 ± 0.00	0.6 ± 0.77	0.6 ± 0.56	-	100
Apr	6	62.0 ± 2.36	14.0 ± 2.64	2.6 ± 0.20	L	32.8 ± 5.6	0.9 ± 0.24	6.9 ± 1.81	1.0 ± 0.75	+	100
May	5	55.2 ± 0.70	12 ± 1.41	2.8 ± 0.40	L	40 ± 0.00	1.6 ± 0.56	3.0 ± 0.70	1 ± 0.07	+	100
	1	50 ± 0.00	17 ± 0.00	1.0 ± 0.00	I	17.0 ± 0.00	1.1 ± 0.00	- - - -	1.0 ± 0.00	-	100
Jun	3	65 ± 4.58	- - - -	- - - -	I	30.7 ± 3.23	1.2 ± 0.32	2.5 ± 0.11	1.5 ± 1.30	-	100
Jul	5	55.8 ± 1.92	27 ± 13.58	1.2 ± 0.27	I	29 ± 6.14	1.4 ± 0.35	5.9 ± 1.07	1.1 ± 0.05	+	75
										-	25
Aug	3	59.7 ± 1.83	27.5 ± 17.67	1.0 ± 0.05	I	28.2 ± 1.13	1.0 ± 0.07	4.7 ± 1.27	1.05 ± 0.07	-	100
Sept	–										
Oct	–										
Nov	–										
Dec	4	63.5 ± 6.14	12.3 ± 1.52	3.6 ± 0.27	L	37.8 ± 1.02	1.3 ± 0.10	27 ± 23.81	2.0 ± 0.05	+	100

Table 13. Condition of gonads of subadult female Aneides ferreus in every month except January. Individuals are placed in order by general size (S-V) length and the state of development of the oviducts, i.e., I = Intermediate; E = Young. All measurements are in (mm) and standard deviations are given.

Month	N	State of development	$\bar{x}$ Length (S-V)	Oviduct $\bar{x}$ length	Oviduct $\bar{x}$ width
Jan	–				
Feb	8	I	34.9 ± 4.92	10.1 ± 4.42	0.2 ± 0.31
Mar	10	I	34.5 ± 3.87	12.2 ± 5.51	1.1 ± 1.4
	12	E	24.0 ± 2.50	4.7 ± 1.37	0.2 ± 0.19
Apr	8	I	46.0 ± 4.24	10 ± 0.00	0.5 ± 0.07
	25	E	23.2 ± 9.01	5.5 ± 1.80	0.2 ± 0.36
May	7	I	39.0 ± 9.97	16.4 ± 10.4	0.2 ± 0.00
	4	E	25.4 ± 3.63	5.5 ± 0.91	0.3 ± 0.55
Jun	1	I	47.5 ± 0.00	12 ± 0.00	1.5 ± 0.00
	1	E	32 ± 0.00	10 ± 0.00	0.9 ± 0.00
Jul	19	E	30.7 ± 10.99	9.8 ± 5.38	0.7 ± 0.18
Aug	2	I	38.0 ± 6.08	8.4 ± 2.89	0.4 ± 0.07
	8	E	26.3 ± 4.37	4.5 ± 1.32	0.2 ± 0.16
Sept	2	I	41.0 ± 2.82	10.3 ± 0.28	0.7 ± 0.00
Oct	2	I	38.5 ± 2.12	8.6 ± 4.80	0.7 ± 0.42
Nov	4	I	44.2 ± 5.26	9.4 ± 3.25	0.7 ± 0.23
Dec	2	I	46.0 ± 7.07	11.1 ± 0.14	0.4 ± 0.49
	10	E	34.8 ± 4.32	8.9 ± 1.58	0.5 ± 0.14

examined, but no histological sectioning of cloacae was attempted. Within the cloacae the spermathecae were abundantly pigmented with melanophores, causing them to appear black.

The number of eggs present tended to be inversely proportional to size of the eggs. The late stage of egg development (2.6 to 3.6 mm in diameter) was characterized by a smaller mean number of eggs (11 to 18). The intermediate stage in egg development (1.0 to 2.2 mm in diameter) had a mean number of eggs ranging from 6.5 to 27.5. The early stage in egg development ranged from 0.2 to 0.5 mm in diameter while the mean number of eggs ranged from 41 to 50 (Table 12).

As expected, the mature females with the eggs in later stages of development also exhibited the longest mean length and width of oviduct. One exception to this was an animal with an intermediate stage of egg development and an oviduct 49 mm long (Table 12).

Individuals in the late stage of egg development contained sperm in the spermatheca in February, March, April, May, and December while animals in the intermediate stage of egg development showed sperm present in February, March, and July. No representatives of the early stage of egg development had sperm present in the spermatheca.

Table 13 shows the oviduct length and width of female subadults to be generally smaller than those of even the early stage of egg development in the mature group (Table 12). These subadult individuals displayed either no eggs or eggs of the very early stage of development. No analysis of spermathecae was attempted in this group and they were classed by development of oviduct and snout-vent length alone.

Survivorship

The results of the records kept on animals maintained under laboratory conditions showed a differential pattern of death. Of the total number of individuals dying (157) during the course of the study (February, 1968 to July, 1969), 100 or 63.7% were of the young size class, while 32 or 20.4% belonged to the immature group. Twenty-five adult animals (15.9%) died during this period (Table 14).

Table 14. The number of deaths of Aneides ferreus occurring under laboratory conditions during the time period of February, 1968 to July, 1969.

	Size class		
	Adult	Immature	Young
Number of dead	25	32	100
% of Total deaths	15.9	20.4	63.7

A restatement of what was meant by laboratory conditions is timely at this point. All animals were maintained in covered polystyrene boxes, the bottoms lined with a folded paper towel, wetted with distilled water. No more than 12 animals were maintained together per box and the boxes, containing animals, were housed in a darkened incubator at 15 ^{o}C. Animals were fed vestigial-winged *Drosophila* twice a week and the boxes were cleaned once a week.

Survivorship of *Aneides ferreus* was therefore dependent upon the conditions outlined above and on the varied conditions of experimental trials in habitat selection; measurement of respiration; and handling for color descriptions, sexing, and body measurements.

DISCUSSION

Habitat Preference

The initial studies performed on this problem were concerned with the selective pattern of habitat types at room temperature (22°C) and utilized animals collected from an urban location. Results showed a significant difference in choice of litter types, as bark litter was a preferred habitat type by the three size classes involved (Table 4; Figure 3). This particular group displayed a rather convincing pattern of selection, indicating that individuals from a somewhat atypical urban environment have maintained what might be considered a natural pattern of selection, that of bark litter. The point should be stressed that the surroundings where these individuals were collected was not what is usually considered natural habitat by previous descriptions (Stebbins, 1954a, 1966; Storm, 1948). There was a high density population (311 animals collected) in a restricted habitat of scattered rocks, concrete, boards, and a wet rug. It is noteworthy that over 40 individuals were taken from the cover and folds of the wet rug with most of these animals of the young and immature size classes. It seems reasonable to conclude that the high selection of bark litter displayed by this experimental group at 22°C was due to the shift of all size classes from their "natural" habitat to the substitute choice of the bark litter (Figure 3).

These results of the preliminary investigations further illustrate the adaptability of <u>Aneides ferreus</u> and provide insight to the success of this plethodontid.

Upon analysis of the effect of temperature (10, 20, and 25 ^{o}C) on the selective pattern of <u>Aneides ferreus</u> collected from a number of locations (Table 1), it was apparent that bark litter continued to be selected in preference to rock litter or leaf litter in Group I animals (Table 3).

When these results are related to the findings of the trials at room temperature (22 ^{o}C) there is a positive correlation of findings. This would seem to indicate that the individuals from the urban collecting site (rocks, concrete, boards, and wet rug) continued to select bark litter at different temperatures.

Regarding the relationships between the selective preference of each size class (adult, immature, and young) and the type of litter selected at different experimental temperatures, the results become more varied and less clear-cut in trials with Groups II and III.

Adult members of Groups II and III selected rock litter over bark litter as the temperature increased. The trend seemed to indicate that animals of the adult size class, whether collected from mixed rock, bark, and boards; rock only; or bark and boards only, tended to show a preference for rock at higher temperatures.

In general, the adult pattern for selection of leaf litter was without a definite trend other than not being an attractive choice. What these trends in selection of habitat type by adults seem to indicate is that this size class has less preference for specific habitat types and therefore could be found in nature in either a bark or rock habitat but probably not in leaf litter. Such findings might be explained by a study of Aneides aeneus (Gordon and Smith, 1949) where it was found that the type of rock was not the limiting factor in habitat choice but rather the type of crevices formed by the rock (depth and position).

A similar pattern is probably also true with adult Aneides ferreus in that their occurrence in natural bark and rock habitat is not so much dependent upon the composition of litter (bark or rock) but on the condition of the habitat (depth and position). It appears reasonable to assume that adult animals could be found with equal success in either a bark or a rock habitat, given suitable conditions for habitation in each habitat.

Perhaps this less specific requirement for habitat type, by adults, would prove to be beneficial for survival of the species by allowing courtship, breeding, and laying of eggs in any suitable habitat. This ability would also tend to act as a means of dispersal while limiting intraspecific competition.

In support of this reasoning is the knowledge that the annual cycle of Aneides aeneus involved a close association with the rock habitat and consisted of (1) the breeding period, (2) the dispersal and aggregation period, (3) the hibernation period, and (4) the post-hibernation aggregation and dispersal period (Gordon, 1952). The activity of each of these periods revolved about two types of rock crevices, tending to spread the population and provide a "buffering effect" against high temperatures and drying.

The results of habitat experiments conducted with immature animals (Group I) show a significantly higher preference for bark litter at the three trial temperatures, as was also true in the adults of the same group. There is no plausible explanation as to why this experimental group displayed no selection of leaf litter as it was selected in all other experimental trials. The litter used was of the same type (oak and maple) throughout the course of the study.

There was a general trend for Group II and III animals to select rock litter over bark litter as the temperature increased to 25°C. The selective preference of rock litter over bark litter by Group II B individuals collected in bark would indicate that the immature size class of Aneides ferreus is not as selective as the young size class, but also not as wide in selective nature as the adults, and is intermediate for this pattern. Analysis of the results of immature Group III individuals provides further indication of their intermediate selective nature (Figure 8 A-C).

It seems reasonable to assume that the increased selection of leaf litter at 25 $^{\circ}$C resulted when the choice between bark litter and rock litter became equalized between members of this experimental group. Some of the individuals that had selected rock and bark at lower temperatures tended to shift to leaf litter at this high temperature (Figure 8 A-C).

The most striking pattern of selective preference of habitat type was displayed by the young size class. In every trial, bark litter was a preferred selection over rock litter or leaf litter. This is an indication that young Aneides ferreus are much more selective and persistent in their choice of habitat type than the adult or immature individuals. These findings differ from the activity of recently hatched salamanders (Ensatina), which upon reaching the surface for the first time, seek cover under any litter encountered if conditions are not unfavorable there (Stebbins, 1954b). In comparison, the Aneides ferreus hatched and raised under laboratory conditions, displayed selective preference for bark litter, not unlike the field-collected animals.

There is reason to suspect that these findings support the above (adult and immature) results, indicating that as Aneides ferreus undergoes maturation it likewise changes selective preference of habitat type, i.e., a high preference for bark litter (young size class); an intermediate preference between bark litter and rock litter (immature size class); a wide selective preference between bark litter and rock litter (adult size class).

These experiments have thus provided a positive answer to the proposed question of whether or not there is a selective pattern of choice of habitat type and has further shown preference patterns of different size classes at different temperatures.

Respiration Studies

It is well known that the rate of respiration of poikilothermous animals changes directly with temperature (Vernberg, 1952). The results of the present investigations demonstrate clearly that *Aneides ferreus* is typical in this respect (Table 6; Figure 9).

The effect of feeding and nonfeeding was not clear-cut at 10°C but became more evident at 20 and 25°C, i.e., with higher rates of respiration.

Figure 9 shows at 10°C a relatively low rate of oxygen consumption by the adult and immature size class in both fed and nonfed groups. This lack of significant difference was probably due to the low temperature where respiration was at such a minimal level that the effect of size (at least in this size range) and/or feeding was not apparent. Vernberg (1959a, b) showed in fiddler crabs (*Uca*) that starvation caused an initial decrease in metabolic rate followed by a relatively constant rate. It is possible that in the present experiment, at 10°C, this decrease was so slight that it was nondetectable. With increased temperature (20 and 25°C) there was a marked increase in respiration rates, resulting in rather clear-cut differences between fed and nonfed individuals. The young animals did show a higher mean respiratory rate at the low temperature (10°C), tending to support the general observation that small animals exhibit a higher standard metabolic rate than related animals of greater body weight (Vernberg, 1952).

The adult size class (fed and nonfed) displayed a similar rate of respiration at 20°C. This pattern of nearly equal respiration rate between fed and nonfed was again apparent at 25°C.

It seems reasonable that with an increased size and therefore a lower rate of respiration, the adult size class did not reach a metabolic level, under the three trial temperatures, where feeding or not feeding affected their metabolism. In support of this reasoning were the results of several respiratory measurements of gravid females. There was no apparent difference between respiratory rates of these individuals and other adults. At these relatively lower rates of respiration the feeding regime and breeding condition seem not to affect oxygen consumption in heavy, adult <u>Aneides ferreus</u>.

It is clear (Figure 9) that the immature size class displayed a significant increase in oxygen consumption between 10 and 20°C. The fed individuals demonstrated the greater increase, a pattern continuing at 25°C with the fed animals again displaying the higher respiration rate. The young fed animals exhibited the most obvious effect of feeding upon respiration rates with a significantly higher rate at each of the higher temperatures. Why the nonfed members of this size class showed a relatively slight increase in respiration rate at 20°C was a mystery, especially when comparing the rates with those of the immature at the same temperature; the slight shift upward in the nonfed young was overshadowed by the pronounced increase by the nonfed immature group.

From an analysis of the data presented it is possible to draw the following conclusions about oxygen consumption in Aneides ferreus: 1) Young individuals tended to show the highest respiratory rate and also generally the greatest standard deviation about the mean respiration rate. There was a significantly higher respiratory rate among fed individuals than those nonfed, at 20 and 25°C; 2) immature animals showed an intermediate respiratory rate between young and adult classes at 20 and 25°C. Generally, the standard deviation about the mean respiratory rate was also intermediate. Fed immature animals displayed higher respiratory rates than nonfed individuals at higher temperatures; 3) adult animals displayed the lowest respiratory rate with the lowest standard deviation about the mean. This size class exhibited the least variation in respiration rate between fed and nonfed individuals at all three temperatures.

Color Patterns

A detailed analysis of the dorsal and ventral color patterns of adult, immature, and young *Aneides ferreus* lead to the following remarks.

The adult animals typically displayed a deep brown dorsal color and a slate or light brown venter especially when collected in a bark habitat. This is the typical dark phase of the salamander as described by Stebbins (1954a).

Observations of *Aneides ferreus* made under laboratory conditions supported the description of captive *Batrachoseps* kept at room temperature in that under these conditions animals tended to loose the lipophore reds and yellows and to assume a more or less blackish condition (Hendrickson, 1954). Further, when *Aneides ferreus* from any habitat were maintained at 15°C they assumed the same blackish condition.

In more specific terms, the surface of the snout of adults was typically finely sprinkled with golden flecks, therefore appearing dark due to the relative lack of iridophore invasion (Category I), while the dorsal surface of the body was flecked in golden patterns. The apparent leucophore color of the adults ranged between yellow and white, while blue leucophores were rare. Based on these observations, an investigator would not often err in placing animals into the adult size class by the lack of blue leucophores alone (Table 7).

Immature individuals were categorized by the higher concentration of iridiphores, mostly golden, on the snout surface (Category II), and by the addition of coppery (reddish) color to the dominant golden patterns of the dorsal surface. In comparing adults and immatures with respect to the leucophore color, it becomes apparent that white coloration was nearly the same in each size class. There was a striking increase of blue coloration in the immatures coupled with a scarcity of yellow coloration. The reverse was true in the adults, adding further to the speculation that a means of classifying individuals into size groups by the presence or absence of blue leucophores is possible (Table 7).

Young animals tended to be the most colorful class described, due to the high concentration of coppery or golden iridophores on the snout (Categories II and III) and by the prevalence of coppery and golden dorsal color patterns. In final support of a classification scheme based upon leucophore coloration was the 100% occurrence of blue leucophores in this size class.

Upon analysis of the ventral melanophore network, in relation to size class, an obvious trend away from a dark venter due to a tight continuous network of melanophores (young) to a lighter ventral pattern resulting from close set but separate melanophores (adult) was noted. These findings seem to indicate less variation in melanophore pattern and distribution than was found in Plethodon jordani jordani, where concentration of melanophores tended to make the chins lighter than bellies (Reynolds, 1959).

Aneides ferreus also displayed an opposite trend in ventral melanophore infiltration from Plethodon jordani melaventris where darker venters were found in larger individuals (Gordon, 1960).

Upon analysis of the results obtained from the description of leucophores present in sites located at five transverse ventral sections (Figure 2 A-B), another clear trend was established, i.e., with maturation from young to adult there was a decrease in leucophores present per descriptive site (Table 8).

The high standard deviation found in the number of leucophores present in the descriptive sites of adults resulted from the presence of the high number of sites that had no leucophores (zero) and the occurrence of a few that displayed as high as seven leucophores.

A clear explanation as to the occurrence of the five (aberrant) light colored animals is lacking; however, when their color descriptions were compared with those of Storm (1948) regarding three Aneides ferreus collected in a similar habitat, it seems possible that the rather atypical habitat (steep shale outcrops) may be correlated with the aberration. Whether this form of Aneides ferreus is an example of a polymorphic population will not be known without future investigations.

In summary, it appears possible to categorize individual Aneides ferreus into three size classes (adult, immature, and young) by means of coloration alone as follows: 1) Adults - dark snout surface finely sprinkled with golden flecks and a dorsal surface flecked in golden patterns accompanied by yellow and/or white leucophores. A relatively light colored ventral pattern of melanophores with few leucophores present; 2) immature - a snout surface invaded quite extensively by mostly golden iridophores and golden or coppery patterns of color with blue or white leucophores on the dorsal surface. A relatively dark ventral surface with a more broken melanophore network and several leucophores present; 3) young - a snout surface heavily invaded by coppery or golden iridophores and a dorsal surface of golden or coppery colors concentrated into patterns always accompanied by blue leucophores. A dark venter composed of a rather tight continuous network of melanophores and many leucophores.

Breeding and Early Life History

The results of observations made on spermatophore deposition, transfer of the sperm cap to the female, hatching of eggs, and initial feeding by young have contributed to the understanding of the life cycle of *Aneides ferreus*.

From observations of animals maintained under laboratory conditions at 15°C, it was possible to time and describe events in the breeding and early stages in the life cycle of the clouded salamander. The male spermatophore deposition took place in early May (6th and

8th), followed by the female pick-up of the sperm cap, probably on the same day as deposition. It was believed that, as in Ensatina, absorption of the sperm cap required only a few days in captive animals (Stebbins, 1954b). It was also shown that a single female can pick up more than one sperm cap following spermatophore deposition. This would no doubt be beneficial to the species by increasing the probability of insemination. There appeared to be a clean transfer of the sperm cap as demonstrated by the lack of sperm on the anterior tip of the jelly stalk.

The size of the spermatophore jelly stalk compared favorably with the size of the jelly stalk of eastern plethodontids, Plethodon jordani metcalfi (Organ, 1958) and Plethodon glutinosus (Organ, 1960a).

The general time of egg deposition of four females corresponds with the date reported by Storm (1947). From these dates it would appear that eggs are deposited in late June and throughout the month of July. The mean number of six eggs deposited per clutch during this study was fewer than the nine reported by Dunn (1942) or the mean number of 13 observed by Storm (1947). A possible explanation for the smaller clutch size in this study is related to the conditions within the laboratory. The 15°C temperature was probably lower than the temperatures experienced during a comparable period in nature. This would tend to affect the feeding regime of the females; perhaps several eggs were eaten before they could be observed or egg absorption took place in hungry females before the time of deposition.

It can be concluded from the results obtained in the attempted incubation and hatching of eggs that the presence of the female is necessary in the retardation of mold. Speculation as to the role of the female in this respect has ranged from the production of inhibitory skin secretions to an active massage of the eggs. Perhaps future investigations will reveal the answer.

Gordon (1952) reported evidence that the eastern plethodontid, _Aneides aeneus_, does not leave the eggs to search for food and therefore does not eat during the three months of guarding. This substantiates the observations of the guarding behavior of _Aneides ferreus_ in the present study and differs from the behavior of _Ensatina_, where the female may from time to time leave the eggs in search of food and replenishment of body water (Stebbins, 1954b).

The duration of the incubation process was about three months (87 days), a period somewhat longer than the 65 days reported by Storm (1947). This increased incubation period was probably due to the lower temperature (15°C) at which the eggs were maintained, as opposed to the room temperature in Storm's (1947) report. Considering these points, eggs developing in nature probably are affected by the environmental temperature, but generally hatch in approximately two months.

From the observations of the newly hatched young, it can be assumed that initial feeding takes place about two weeks after hatching, following the period of gill absorption and utilization of yolk sac material.

Newly hatched animals were very active and exhibited a mean length of 25.9 mm. In his work on Batrachoseps, Hendrickson (1954) found no salamanders that could be definitely called newly hatched, and only very few that were "new of the year." In the present investigation about 25 animals were collected or raised that were believed to be "new of the year."

In summary, the events of breeding and early life history of Aneides ferreus are as follows: 1) Spermatophore deposition in early May; 2) egg deposition in late June and throughout July; 3) hatching of eggs in late October (probably earlier in nature); 4) first feeding of young about two weeks after hatching or early November in this study.

Reproductive Studies

Generalizations regarding the reproductive cycle of Aneides ferreus are presented with the knowledge that animals were not available from every month of the year and the number of individuals for each period was at times limited.

Results indicated that even small (24 mm snout-vent length), immature individuals could be sexed by means of the presence of cloacal papillae in males and cloacal folds in females.

Dissections and microscopic examination revealed the composition and development of the reproductive organs in mature and subadult animals. The following are conclusions drawn from these examinations.

Reproductive Cycle in Males

There was little variation in the appearance of the testes and vasa deferentia of mature males within any monthly sample or throughout the year.

The presence of sperm in the vasa deferentia in February through August (Table 10) supports the laboratory observations of the time of spermatophore deposition in early May. It is probable that the most active courtship and breeding period of the clouded salamander is during May and June. However, it seems quite possible that there is a prolonged breeding period. Tables 10 and 11 show that western Oregon males may have sperm in the vasa deferentia at almost any time of the year, probably depending on locality and environmental conditions. Table 12 shows mature females in December with late developed eggs and sperm in the spermatheca. These are indications of a prolonged breeding period similar to that of *Ensatina* (Stebbins, 1954b) and *Plethodon glutinosus* (Highton, 1962).

It seems unlikely that male individuals below 36 mm, snout-vent length, would be reproductively active. From data obtained from analysis of the growth patterns of young individuals raised in the laboratory and from collection records, it seems probable that the time of reproductive maturity generally occurs during the second year, varying somewhat with environmental conditions. This two-year figure would be consistent with that reported for Plethodon cinereus of the eastern United States (Test and Bingham, 1948) and somewhat less than reported for some Ensatina of the west (Stebbins, 1954b).

Reproductive Cycle in Females

Results shown in Table 12 indicate that the egg size in mature Aneides ferreus in the late stage of egg development tended to be smaller than those reported for Ensatina, but the number per individual was nearly the same, i. e., about 12 (Stebbins, 1954b).

Storm (1948) reported 14 ovarian eggs with a mean diameter of 3 mm from an animal collected in February. These data are quite similar to the findings in the present studies where mature females in February had a mean number of 11 eggs (late stage of development) that measured 2.7 mm in diameter.

Upon examination of dissected females it was evident that the subadult individuals or the mature animals in the early stage of egg development were alike in having thin, straight oviducts. The oviducts of mature females of the late stage of egg development were convoluted and thick-walled.

Table 12 shows that generally sperm were present in the spermatheca during months when individuals contained eggs in the late stage of development and were sometimes lacking in those with eggs in the intermediate stage of development. Sperm were totally lacking in individuals of the early stage of egg development.

It seems improbable that eggs could develop from the early stage to the mature, late stage in a single season. An illustration of the time involved in the maturing of eggs was the observation made upon females collected in February that had well-developed ovarian eggs visible through the abdomen. Nevertheless, when these animals were brought into the laboratory they did not lay eggs for about five months. It seems very likely that it takes two years to accumulate sufficient yolk to be considered a late stage of development. The mature females would then undergo egg deposition every other year, unlike the yearly egg laying of Plethodon glutinosus (Highton, 1956).

The smallest sized female with intermediate developed eggs was 50 mm (snout-vent length). This individual, collected in May, lacked sperm in the spermatheca and even if it was able to pick up and retain sperm in a viable condition, the eggs would require a year more to mature. Therefore, it is concluded that the first egg deposition by females is in their third year of life.

Egg deposition in nature is probably in late June and throughout

July. In support of this were the observations of egg deposition under laboratory conditions during this time and also the dissections which revealed no large eggs of a diameter of 2.8 mm or more immediately after June. The only eggs present in July and August were of an intermediate developmental stage.

Survivorship

It has been shown, from a review of the number of deaths occurring under laboratory conditions, that there was a differential death pattern, inversely proportional to the size (age) of individuals (Table 14).

The young *Aneides ferreus* showed the highest mortality, followed by the immature, and adults, in that order. This pattern seems reasonable when one considers that the young size class of many animals tends to be the most susceptible to mortality. The survivors to advanced age die at a lower and comparatively constant rate (Deevey, 1950).

In studies of the population dynamics of the plethodontid salamander, *Desmognathus*, Organ (1961) demonstrated a trend of survivorship in males of this species analogous to that described for *Aneides ferreus*.

Based upon laboratory and field observations, a logical explanation for the greater percentage of death in the young size class involved injuries sustained in collecting and handling. An equally important factor was related to the greater surface to volume ratio of this size class. The young tended to dehydrate much faster than the immature or adults.

SUMMARY

Aneides ferreus, the clouded salamander, was utilized in laboratory investigations designed to measure aspects of its ecology.

Animals from western Oregon were shown to exhibit a significant pattern in selection of litter types. Adult, immature, and young size classes displayed different selective preferences at different temperatures. Generally, the young selected bark litter; the immature were intermediate in preferences between bark litter and rock litter, and the adults exhibited no marked preference between bark litter and rock litter.

Oxygen consumption was used as a measure of respiratory rates at different temperatures (10, 20, and 25°C) and under different feeding regimes. The young showed consistently the highest respiratory rates and fed individuals were higher than nonfed. The immature group was generally intermediate between young and adults, with fed individuals showing greater respiratory rates at 20 and 25°C than nonfed. Adult animals had consistently the lowest respiratory rates with no significant differences between those fed and nonfed.

Population characteristics were analyzed by descriptions of dorsal and ventral color patterns. Adults were generally dark with flecks of gold on the dorsum while their venter was light colored, with few leucophores present. Immature animals displayed mostly golden or coppery iridophores on the dorsal surface and a broken melanophore

pattern on the venter. The young were coppery or golden with blue leucophores on the dorsum and the venter was dark due to a continuous network of melanophores.

Animals were sexed by analysis of the inner vent and dissection revealed mature males had little annual variation in size and appearance of the testes and vasa deferentia. September was the only month analyzed where sperm were not found in the vasa deferentia. Males below 36 mm snout-vent length are considered immature. Males probably mature during the second year after hatching.

Sperm were found in the spermathecae of females during every month that eggs of the late stage of development were present.

Eggs are probably deposited every other year and only after the female is three years old and approximately 55 mm in snout-vent length.

A prolonged breeding period is suspected with deposition of the spermatophore commencing in early May. Eggs are deposited in late June and through July with an average clutch size of about 12. Hatching occurs about two months following egg deposition with the newly hatched animals measuring approximately 26 mm total length. Young live on stored yolk material until the first feeding roughly 14 days after hatching. A differential death pattern inversely proportional to size was noted.

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APPENDIX

Appendix Table 1. Chi-square analysis of the experimental results in habitat selection by three size classes [Adults (A), Immature (IM), and Young (Y)] of Aneides ferreus, during preliminary studies at room temperature (22°C). Individuals were collected in an urban habitat of boards, rock, and wet rug.

Number (N =)	Size class	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
116	A	Rock	20	38.6	17.2	
		Bark	85	38.6	73.3	
		Leaves	11	38.6	9.5	84.5**
75	IM	Rock	10	25.0	13.3	
		Bark	50	25.0	66.7	
		Leaves	15	25.0	20.0	38.0**
240	Y	Rock	49	80.0	20.4	
		Bark	133	80.0	55.4	
		Leaves	58	80.0	24.2	53.2**

**Significant at 1% level.

Appendix Table 2. Summary table of chi-square analysis of experimental results in habitat selection by three size classes [Adults (A), Immature (IM), and Young (Y)] of Aneides ferreus in experimental Group I; individuals were collected in an urban habitat of bark, rock, and wet rug.

Number (N =)	Size class	Trial temp. (°C)	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
126	A	10	Rock	53	42.0	42.1	
			Bark	71	42.0	56.3	
			Leaves	2	42.0	1.6	60.9**
35	IM	10	Rock	9	11.6	25.7	
			Bark	26	11.6	74.3	
			Leaves	0	11.6	0.0	30.1**
141	Y	10	Rock	47	47.0	33.3	
			Bark	87	47.0	61.7	
			Leaves	7	47.0	5.0	68.1**
124	A	20	Rock	44	41.3	35.5	
			Bark	79	41.3	63.7	
			Leaves	1	41.3	0.8	73.9**
33	IM	20	Rock	7	11.0	21.2	
			Bark	26	11.0	78.8	
			Leaves	0	11.0	0.0	32.9**
97	Y	20	Rock	34	32.3	35.1	
			Bark	57	32.3	58.8	
			Leaves	6	32.3	6.1	40.4**

Appendix Table 2. (Continued).

Number (N =)	Size class	Trial temp. (°C)	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
116	A	25	Rock	26	38.6	22.4	
			Bark	87	38.6	75.0	
			Leaves	3	38.6	2.6	97.5 **
27	IM	25	Rock	6	9.0	22.2	
			Bark	21	9.0	77.8	
			Leaves	0	9.0	0.0	26.0**
86	Y	25	Rock	28	28.6	32.5	
			Bark	53	28.6	61.7	
			Leaves	5	28.6	5.8	40.2**

**Significant at the 1% level.

Appendix Table 3. Summary table of chi-square analysis of the experimental results in habitat selection by three size classes [Adults (A), Immature (IM), and Young (Y)] of Aneides ferreus in experimental Group II. Combined results represent animals collected in rock, bark and boards. Rock animals only represent animals collected in rock (Group II A) and bark and board animals only represent forms collected in bark and boards (Group II B). Trial temperature $10^{o}C$.

Number (N =)	Size class	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
		Group II - Combined Results				
183	A	Rock	75	61.0	41.0	
		Bark	60	61.0	32.8	
		Leaves	48	61.0	26.2	6.0*
53	IM	Rock	28	17.6	52.8	
		Bark	14	17.6	26.4	
		Leaves	11	17.6	20.8	9.3**
116	Y	Rock	38	38.6	32.8	
		Bark	58	38.6	50.0	
		Leaves	20	38.6	17.2	18.7**

Appendix Table 3. (Continued).

Number (N =)	Size class	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
		Group II A - Results of rock animals only				
104	A	Rock	46	34.6	44.2	
		Bark	35	34.6	33.7	
		Leaves	23	34.6	22.1	7.6*
13	IM	Rock	6	4.3	46.2	
		Bark	6	4.3	46.2	
		Leaves	1	4.3	7.6	3.8
27	Y	Rock	6	9.0	22.2	
		Bark	17	9.0	63.0	
		Leaves	4	9.0	14.8	10.9**
		Group II B - Results of bark and board animals only				
79	A	Rock	29	26.3	36.7	
		Bark	25	26.3	31.6	
		Leaves	25	26.3	31.6	0.4
40	IM	Rock	22	13.3	55.0	
		Bark	8	13.3	20.0	
		Leaves	10	13.3	25.0	8.6*
89	Y	Rock	32	29.6	36.0	
		Bark	41	29.6	46.1	
		Leaves	16	29.6	17.9	10.8**

*Significant at 5% level.
**Significant at 1% level.

Appendix Table 4. Summary table of chi-square analysis of the experimental results in habitat selection by three size classes [Adults (A), Immature (IM), and Young (Y)] of *Aneides ferreus* in experimental Group II. Combined results represent animals collected in rock, bark and boards. Rock animals only represent animals collected in rock (Group II A) and bark and board animals only represent forms collected in bark and boards (Group II B). Trial temperature 20°C.

Number (N =)	Size class	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
		Group II - Combined results				
189	A	Rock	90	63.0	47.6	
		Bark	65	63.0	34.4	
		Leaves	34	63.0	18.0	25**
55	IM	Rock	24	18.3	43.6	
		Bark	17	18.3	30.9	
		Leaves	14	18.3	25.5	2.9
107	Y	Rock	24	35.6	22.4	
		Bark	64	35.6	59.8	
		Leaves	19	35.6	17.8	34.1**

Appendix Table 4. (Continued).

Number (N =)	Size class	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
		Group II A - Results of rock animals only				
103	A	Rock	53	49.0	51.5	
		Bark	32	49.0	31.1	
		Leaves	18	49.0	17.4	18.0**
19	IM	Rock	7	6.3	36.8	
		Bark	7	6.3	36.8	
		Leaves	5	6.3	26.3	0.4
25	Y	Rock	5	8.3	20.0	
		Bark	12	8.3	48.0	
		Leaves	8	8.3	32.0	2.96
		Group II B - Results of bark and board animals only				
86	A	Rock	37	28.6	43.0	
		Bark	33	28.6	38.4	
		Leaves	16	28.6	18.6	8.7*
36	IM	Rock	17	12.0	47.2	
		Bark	10	12.0	27.8	
		Leaves	9	12.0	25.0	3.1
82	Y	Rock	19	27.3	23.2	
		Bark	52	27.3	63.4	
		Leaves	11	27.3	13.4	34.6**

*Significant at 5% level.
**Significant at 1% level.

Appendix Table 5. Summary table of chi-square analysis of the experimental results in habitat selection by three size classes [Adults (A), Immature (IM), and Young (Y)] of *Aneides ferreus* in experimental Group II. Combined results represent animals collected in rock, bark and boards. Rock animals only represent animals collected in rock (Group II A) and bark and board animals only represent forms collected in bark and boards (Group II B). Trial temperature 25°C.

Number (N =)	Size class	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
		Group II - Combined results				
183	A	Rock	95	61.0	51.9	
		Bark	73	61.0	39.9	
		Leaves	15	61.0	8.2	56**
106	IM	Rock	53	35.3	50.0	
		Bark	43	35.3	40.6	
		Leaves	10	35.3	9.4	28.6**
99	Y	Rock	17	33.0	17.2	
		Bark	62	33.0	62.6	
		Leaves	20	33.0	20.2	38.4**

Appendix Table 5. (Continued).

Number (N =)	Size class	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
		Group II A - Results of rock animals only				
93	A	Rock	50	31.0	53.8	
		Bark	34	31.0	36.6	
		Leaves	9	31.0	9.6	27.6**
14	IM	Rock	9	4.6	64.3	
		Bark	4	4.6	28.6	
		Leaves	1	4.6	7.1	7.0*
17	Y	Rock	3	5.6	17.7	
		Bark	11	5.6	64.6	
		Leaves	3	5.6	17.7	7.5*
		Group II B - Results of bark and board animals only				
63	A	Rock	34	21.0	53.9	
		Bark	27	21.0	42.8	
		Leaves	2	21.0	3.2	27**
77	IM	Rock	38	25.6	49.4	
		Bark	30	25.6	38.9	
		Leaves	9	25.6	11.7	17.5**
65	Y	Rock	12	21.6	18.5	
		Bark	44	21.6	67.7	
		Leaves	9	21.6	13.8	34.8**

*Significant at 5% level.
**Significant at 1% level.

Appendix Table 6. Summary table of chi-square analysis of the experimental results in habitat selection by three size classes [Adult (A), Immature (IM), and Young (Y)] of *Aneides ferreus* in experimental Group III. Individuals were collected in a mixed habitat of rock and bark.

Number (N =)	Size class	Trial temp. (°C)	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
43	A	10	Rock	12	14.3	27.9	
			Bark	27	14.3	62.8	
			Leaves	4	14.3	9.3	19^{**}
51	IM	10	Rock	31	17.0	60.8	
			Bark	17	17.0	33.3	
			Leaves	3	17.0	5.9	23^{**}
17	Y	10	Rock	4	5.6	23.5	
			Bark	12	5.6	70.6	
			Leaves	1	5.6	5.9	11.4^{**}
35	A	20	Rock	21	11.6	60.0	
			Bark	12	11.6	34.3	
			Leaves	2	11.6	5.7	15.5^{**}
37	IM	20	Rock	14	12.3	37.8	
			Bark	22	12.3	59.5	
			Leaves	1	12.3	2.7	18.2^{**}
10	Y	20	Rock	2	3.3	20	
			Bark	8	3.3	80	
			Leaves	0	3.3	0	10.4^{**}

Appendix Table 6. (Continued).

Number (N =)	Size class	Trial temp. (°C)	Trial litter (type)	Observed frequency	Theoretical frequency	% of N	Chi-square value
19	A	25	Rock	10	6.3	52.6	
			Bark	7	6.3	36.9	
			Leaves	2	6.3	10.5	5.2
15	IM	25	Rock	6	5.0	40.0	
			Bark	6	5.0	40.0	
			Leaves	3	5.0	20.0	1.2
11	Y	25	Rock	2	3.6	18.2	
			Bark	5	3.6	45.4	
			Leaves	4	3.6	36.4	1.3

**Significant at 1% level.