SOME ASPECTS OF BODY CHARACTERS, REPRODUCTION, FEEDING, AND PARASITISM OF THE GRAND CANYON RATTLESNAKE, Crotalus viridis abyssus.

by

ROY McENDREE GARRIGUES III
A. B., Kansas Wesleyan University, 1962

A MASTER'S REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY Manhattan, Kansas

1966

Approved by:

Major Professor

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INTRODUCTION

The Grand Canyon rattlesnake, <u>Crotalus viridis abyssus</u> Klauber, 1930, is a relatively unknown rattlesnake which inhabits the remote regions of the Grand Canyon. Although studies and reports on prairie rattlesnakes, <u>C. v. viridis</u>, are numerous, the life history of the Grand Canyon subspecies, <u>C. v. abyssus</u>, has not been thoroughly studied.

This subspecies has been little studied because it is rare and lives in a remote area. Klauber (1938) reports that at no place in the Grand Canyon is $\underline{\mathbf{C}}$. $\underline{\mathbf{v}}$. $\underline{\mathbf{abyssus}}$ abundant, and considerable difficulty has been encountered in obtaining enough specimens to make a careful study. Extremely high temperatures and aridity during the summer months makes it inadvisable to enter the rocky, precipitous, and comparatively inaccessible regions to conduct intensive research for a prolonged period of time.

The purpose of this study was to obtain data on the parasites of this snake and to elaborate on its body characters, food habits and feeding, and reproduction.

STUDY AREA

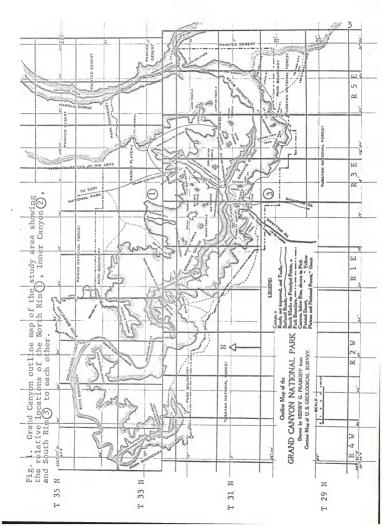
This research was conducted in and immediately around the Grand Canyon of the Colorado River (Fig. 1). Grand Canyon, located in Coconino County, Arizona, just south of the Utah State line and contained within Grand Canyon National Park and Grand Canyon National Monument, contains approximately 1,100 square miles. The canyon is approximately 105 miles long and 1 mile deep.

Lower Sonoran, Upper Sonoran, and Transition life zones are present as shown by vegetation and elevation (U. S. Department of Interior, 1961). Most of the Upper Sonoran and Transition zones consist of inaccessible cliffs while the lower part of the Upper Sonoran and the Lower Sonoran contain benches and desert flats.

The Lower Sonoran zone extends from the bottom of the canyon at 2,000 feet elevation up to about 4,000 feet elevation. The major plants in this life zone were: Utah agave (Agave utahensis)¹/, big sagebrush (Artemisia tridentata), catclaw acacia (Acacia greggi), creosote-bush (Larrea divaricata), common mesquite (Prosopis juliflora), Mormon tea (Ephedra torreyana), ocotillo (Fouquieria splendens), yucca (Yucca spp.) and netleaf hackberry (Celtis laevigata var. reticulata). The common cattail (Typha latifolia) and Fremont cottonwood (Populus fremontii var. fremontii) were present and abundant around all permanent water in this zone.

The Upper Sonoran zone extends from the top of the Lower Sonoran zone to about 7,000 feet in elevation. Dominant plants in this zone are: cliffrose (Cowania mexicana var. stansburiana), one-seed juniper (Juniperus monosperma), Utah juniper (J.

¹Common and scientific names of plants follow Kearney and Peebles (1960).



osteosperma), and pinyon pine (Pinus edulis).

The transition zone extends from approximately 7,000 feet to 8,000 feet elevation. Dominant plants are ponderosa pine (<u>Pinus ponderosa</u>) and quaking aspen (<u>Populus tremuloides</u>).

Rodents are the dominant form of animal life in all life zones of the study area,

The entire canyon is arid, the main source of water being the Colorado River. Most creeks and springs are intermittent. U. S. Department of Interior (1962) records show annual precipitation for the canyon varies from 25 inches on the North Rim to 16 inches on the South Rim, and 10 inches in the Inner Canyon.

Annual average temperatures are $43^{\circ}F$ for the North Rim, $50^{\circ}F$ for the South Rim, and $69^{\circ}F$ for the Inner Canyon. Jurwitz and Kangieser (1959) and the U. S. Department of Interior (1962) give South Rim mean maximum temperatures of $41^{\circ}F$ in January and $85^{\circ}F$ in July while the mean minimum is $17^{\circ}F$ in January and $55^{\circ}F$ in July. North Rim mean maximum temperatures are $44^{\circ}F$ and $81^{\circ}F$ during January and July, respectively, while the mean minimum is $25^{\circ}F$ in January and $62^{\circ}F$ in July. Inner Canyon mean maximum temperature readings vary from $47^{\circ}F$ in January to $105^{\circ}F$ in July, while the mean minimum is $32^{\circ}F$ in January and $73^{\circ}F$ in July.

METHODS

Specimens and data for this study were acquired by personal collection and observation, collection by employees, rangers, and naturalists of the National Park Service, and from study of the C. v. abyssus specimens of the Grand Canyon National Park research museum. Collecting was conducted throughout the Grand Canyon, on the immediate rims, and in Grand Canyon National Monument, from June 6, 1962 to September 14, 1963.

Coloration, body blotches, tail rings, rattles, and scalation were observed and/or counted directly on the live or preserved specimens. The results were then stratified according to sex for the 29 specimens. Scale counts were by rows, being counted on a transverse line from row one on the left side to the last row on the right side. Ventral scales were counted by starting with the first ventral scale under the chin and continuing back. Body and tail lengths were measured in millimeters.

Live specimens were not anesthetized prior to measurement since they were less prone to contract their muscles in the conscious state. External length and internal organ measurements were not determined for preserved specimens because of variations in preservatives and duration of preservation. The preservatives dated back to 1928, and consisted of either 40 percent methyl alcohol or 20 to 70 percent formalin solutions.

The oviducts of all female snakes studied were bisected and examined for the presence of embryos. To compare lengths between gravid and nongravid oviducts, the female snakes were opened along a midline from chin to vent. Each oviduct was measured and compared on the basis of the number of ventral scales that covered it.

Venom toxicity studies were conducted by direct observations

and examinations of the prey following the intramuscular injections of the venom by syringe or fang. Venom for the artificial injection was obtained by milking four captive specimens.

The pH measurements of venom were obtained by using Hydrion Short Range pH Papers $(\!A\!\!)$.

Food habits were determined by examining the entire digestive tract of each snake. The food specimens collected were then identified by teeth and/or hair remains, unidentifiable samples being recorded as such. Food samples for the captive snakes were identified prior to ingestion. Fecal contents were analyzed by careful examination of each sample with the aid of a dissecting microscope. Following ocular examination, each sample was placed in 10cc of deionized water for 8 to 10 hours and the relative pH of the samples determined.

Fecal smears were used in the examination of feces for protozoan cysts and helminth eggs. Small pieces of feces were soaked in a 10 percent physiological saline solution and smeared on a slide, covered with a cover-slip, and examined microscopically at 100X. Another method used to analyze fecal samples was a simplified $NaNO_2$ centrifugal floatation technique combined with the Clayton-Lane method. Ten grams of feces were mixed with 300 ml of distilled water in an Erlenmeyer flask, placed in an Oster blender for $1\ 1/2$ to $2\ \text{minutes}$ and then strained. The fluid was poured into two test tubes (15 ml each) and centrifuged at 2,500 rpm for 3 minutes. All supernatant liquid was decanted and the remaining solid was mixed with a saturated solution of NaNO2. The two test tubes were filled to a meniscus and returned to the centrifuge. A 22 mm square cover-slip which was held in place by adaptive prongs was then placed on top of each test tube and centrifuged at 2,500 rpm for 1 minute. The cover-slips were then

removed, placed on slides, and microscopically examined at 400X.

Examination for internal helminths involved removing the entire digestive tract, bisecting it, and examining it with the aid of a dissecting microscope.

Cestodes were removed by excision from the intestinal epithelium and then examined under low power magnification, classified, and placed in Ward's fixative. Three tetrathyridia were removed from the lumen of two snake's intestines and placed in lacto-phenol for 10 to 14 days for clearing and mounting in balsam.

An adult nematode was removed from the stomach of one snake and stored in 100 percent glycerine.

A single hypertrophied liver and numerous intestinal mesentary cysts were embedded in paraffin, sectioned at $5\,\mu$, stained with Harris haematoxylin and eosin and mounted in a 60 percent solution of synthetic resin.

The spiracular plates were removed from a <u>Cuterebra</u> larva, mounted in balsam, and the remainder of the larva was injected with and stored in 70 percent alcohol.

NATURAL HISTORY AND LITERATURE REVIEW

The Grand Canyon rattlesnake, <u>C. v. abyssus</u>, has been found in the Grand Canyon of the Colorado River, its side canyons, and up to and on both the North and South Rims (Curran and Kauffeld, 1937; Klauber, 1956; Wright and Wright, 1957; Ditmars, 1962). The snake, a rock dweller, was the only poisonous reptile confined to the Grand Canyon (Schmidt and Davis, 1941; Klauber, 1956). The type specimen was collected in the Grand Canyon in 1929. Two related subspecies, <u>C. v. concolor</u>, and <u>C. v. lutosus</u>, were incidental inhabitants in the Grand Canyon. Klauber (1956) and Wright and Wright (1957) found that <u>C. v. abyssus</u> intergraded to the south of Grand Canyon with <u>C. v. nuntius</u>.

Body color of <u>C. v. abyssus</u> was vermillion, salmon, or almost orange, and the body blotches tended to disappear as the snakes matured (Schmidt and Davis, 1941; Wright and Wright, 1957).

McKee (1930) and Schmidt and Davis (1941) found the dark blotches of young <u>C. v. abyssus</u> to be similar to those of the prairie rattlesnake, <u>C. v. viridis</u>. Body blotches were diamond, subrectangular, or elliptic, with serrated edges and numbered 36 to 48, averaging 41.9 (Klauber, 1956). Wright and Wright (1957) found the blotches to be 8 to 12 scales horizontally, and two to five scales longitudinally, with blotch intervals two scales wide. Dorsal blotches were brown with dark borders. A lateral row of brown spots were on the third and fifth scale rows, opposite those on the dorsal side. At the tail, dorsal and lateral spots joined to form transverse bars which shaded from brown to black.

Male tail rings were 7 to 12 in number, averaging 8.2, females having six to eight, averaging 6.9 (Gloyd, 1940; Klauber, 1956). The basal rattle segment was usually black, with the others fading to pink or cinnamon-pink (Wright and Wright, 1957).

The top of the head was light brown and the upper labial area was pink. A faint, light colored postocular stripe was present and light supraocular cross bars (when present) had parallel or inwardly divergent borders. The underside of the head was cream-white with several inches of the neck being cinnamon-pink. Two or three rows of lateral scales and the edges of the ventral scales were onion skin pink, changing to buff-pink or tan on the back. Ventral scales of the tail were pink to tan.

The iris of the eye was brown.

Adult body length seldom exceeded 875 mm and Klauber (1956) placed the average length of the young snakes at 260 mm. His smallest specimen was 250 mm in length. The smallest gravid female found by Wright and Wright (1957) was 684 mm, and the largest adult male was 1,000 mm (Klauber, 1956). The largest specimen reported for this subspecies was 1,025 mm (Durham, 1950). This subspecies was average sized when compared with most other rattlesnakes, but small when compared to the eastern diamondback, C. adamanteus. An average specimen of C. v. abyssus was larger than other rattlesnakes of its species.

Sexual dimorphism in this snake was exhibited by tail length. The tail of the male contained the indrawn hemipenis and was longer, thicker, and averaged 7.3 percent of the body length compared to the female whose tail length was 5.9 percent of the body length (Klauber, 1956).

The top of the head was flat, depressed, and except for the supraoculars, covered with numerous small scales. Wright and Wright (1957) and Klauber (1956) reported the scales to be posteriorly keeled except for the first row on each side of the ventral scales which were unkeeled. Scale rows numbered 25 - 25 - 19 (Klauber, 1956), with an entire anal scute (Wright and Wright,

1957). Ventral scales of the tail were in a single row.

Supralabial scales numbered 13 - 18, averaging 15.6, infralabial scales being 14 - 18, averaging 16.1. Subcaudal scales of the male numbered 23 - 29, averaging 25.4; for the female 18 - 24, with an average of 20.6. Ventral scales of the male were 173 - 185, averaging 178.4; for the female they were 179 - 191, with an average of 185. Ventral caudal scales averaged 24 in number for both sexes.

Four scales contacted the rostral between the prenasals, the prenasal touching the first supralabial on each side. Head scales anterior to the supraoculars were 4 - 8, nasals 2 - 2, loreals 1 - 1, preoculars 2 - 2, and the sub and postoculars were 6 - 6 (Wright and Wright, 1957). Klauber (1956) noted that the upper preocular scale was not in contact with the postnasal scale. There were three or more internasals on this and other subspecies of \underline{C} . $\underline{viridis}$, two on all other rattlesnakes.

Klauber noted that sense of smell by the tongue and Jacobson's organs was not only to locate hibernation dens, but also to find females for reproduction. Location of the female was aided by an odoriferous sex attractant which was secreted from the anal gland of the female (Stebbins, 1954). Gravid females were secretive in the summer and only males were active during this period. The young of these ovoviviparous rattlesnakes had a crude parchment-like casing whether being inside or having just been released to the outside of the female's body. An egg tooth on the upper jaw disappeared shortly after birth. The purpose of this tooth was unknown for the egg covering inside the female was more thin and soft than those of oviparous snakes (Klauber, 1956). Hatches have included 6 to 13 young (Wright and Wright, 1957), and the sex distribution was 1.57 males per female (Klauber, 1956).

The snakes killed their prey by biting, releasing, and then waiting for the death caused by a haemotoxin that started a semi-digestive and hemolytic action (Klauber, 1956). With smaller prey the snake may bite it on the neck or body and hold it until the prey dies. Githens (1931) found that venom of $\underline{\mathbf{C}}$. $\underline{\mathbf{v}}$. $\underline{\mathbf{abyssus}}$, like that of $\underline{\mathbf{C}}$. $\underline{\mathbf{v}}$. $\underline{\mathbf{viridis}}$, had the hemorrhagic effect of causing blood vessel walls to become permeable to blood and it inhibited clotting by hemolysis of the erythrocytes and degeneration of fibrinogen. He also found that in minute amounts the venom had slight agglutinizing effects.

Klauber (1956) found the specific gravity of fresh venom from an adult rattlesnake of the family Crotalidae ranged from 1.07 to 1.10. Specific gravity for <u>C. v. abyssus</u> venom was 1.08 to 1.09. He also found that venom from juveniles and adults milked for a second time were below the normal specific gravity by 0.008 to 0.020. Lowering of the specific gravity was caused by a decrease in residual solids, with young and remilked snakes having a less toxic venom than adult snakes not previously milked.

Klauber's (1956) survey listed the minimum lethal dosage of dried venom for 350 g pigeons at 0.06 mg, and Macht (1937) placed it at 0.1 mg for 22 g mice. Spector (1956) listed the LD $_{50}$ for the type species venom at 3.6 mg/kg in mice. The pH for \underline{c} . \underline{v} . $\underline{abyssus}$ venom ranged from 5.6 to 6.2, with a mean of 5.9.

The average dried venom yield was 97 mg per snake (Klauber, 1956). Klauber recorded a maximum yield of 137 mg of dried venom from one specimen.

Rasmussen (1941) and Olin (1961) listed the food sources available in the Grand Canyon for this subspecies of rattlesnake as: the golden-mantled ground squirrel (Citellus lateralis)²/,

 $^{^{2}}$ Common and scientific names of mammals follow Burt (1961).

young rock squirrel (C. variegatus), young whitetailed prairie dog (Cynomys gunnisoni), ord kangaroo rat (Dipodomys ordi), graynecked chipmunk (Eutamias cinereicollis), cliff chipmunk (E. dorsalis), least chipmunk (E. minimus), Colorado chipmunk (E. quadrivittatus), deer mouse (Peromyscus maniculatus), cottontail rabbit (Sylvilagus nuttali), spruce squirrel (Tamiascuirus fremonti), valley pocket gopher (Thomomys bottae) and birds up to the size of a quail. Klauber (1956) examined one C. v. abyssus that contained a lizard of the genus Sceloporus.

Parasitism in <u>C. v. abyssus</u> has not been recorded in the literature but infections in related snakes have been reported. Self and McMurry (1948) found <u>C. atrox</u> and <u>C. h. horridus</u> to have been infected with a tongue worm, <u>Porocephalus crotali</u>, which lived in the snake's lung cavities. Infection with this species of worm ranged from 5 to 158 per snake, and up to 50 percent of the snakes in the hibernation dens were infected. Other rattle-snakes infected by <u>P. crotali</u> were <u>C. b. basilicus</u> and <u>C. v. viridis</u> (Klauber, 1956). In addition, Sambon (1922) found <u>C. durissus terrificus</u>, and <u>C. adamanteus</u> infected with this parasite. Klauber (1956) reported the tongue worm <u>Raillietiella furcocerca</u> in <u>C. tortugensis</u> and Hill (1935) found <u>Kiricephalus coarctatus</u> in <u>C. adamanteus</u>. Other pentastomids reported in rattlesnakes were <u>Pentastomum proboscidium</u> in <u>C. adamanteus</u>, and <u>C. horridus</u> (Leidy, 1884).

Lopez-Neyra and Diaz-Ungria (1958) found <u>C</u>. <u>durissus</u>

<u>terrificus</u> to be infected with the cestode <u>Ophiotaenia crotali</u>
which Wardle and McLeod (1952) considered to be a primative
genus. Loewen (1940) identified <u>Oochoristica gracewileyae</u> in <u>C</u>.

<u>a</u>. <u>atrox</u> and Alexander and Alexander (1957) found <u>O</u>. <u>crotalicola</u>
in both <u>C</u>. <u>v</u>. <u>helleri</u> and <u>C</u>. <u>cerastes latrorepens</u>. Voge (1953)
collected tetrathyridia of <u>Mesocestoides variabilis</u> from the

intermediate host, C. v. oreganus.

Kreis (1938) reported the nematode <u>Kalicephalus implicatus</u> in <u>C. terrificus</u>. Comroe (1948) discovered <u>K. conoidus</u> in <u>C. triseriatus</u>, and Fantham and Porter (1954) found <u>K. macronatus</u> in <u>C. horridus</u>. <u>Physaloptera obtusissima</u> was reported in <u>C. v. oreganus</u> (Morgan, 1943). Other nematodes reported to have infected rattlesnakes have been <u>Capillaria crotali</u> in <u>C. d. durrissus</u> (Viquez, 1933), and <u>C. terrificus</u> (Yorke and Maplestone, 1962), respectively. Yorke and Maplestone listed <u>Ascaridia flexuosa</u> as occurring in rattlesnakes. <u>Ophidascaris labiatopapillosa</u> has been reported in <u>C. m. migrescens</u> and unidentified nematodes were found in <u>C. atrox</u>, <u>C. polystictus</u>, <u>C. p. pricei</u> and <u>C. v. nuntius</u> (Klauber, 1956).

Self (1945) reported a trematode, <u>Neorenifer crotali</u> in <u>C. atrox</u>, and Fantham and Porter (1954) found it in <u>C. horridus</u>. The rattlesnakes <u>Sistrurus miliarius</u> and <u>S. m. barbouri</u> have been infected with <u>Renifer kansensis</u> (Harwood, 1933) and <u>N. glandularis</u> (Byrd and Denton, 1938), respectively.

Protozoan infections in rattlesnakes included the commensal Entamoeba terrapinae (Miller, 1951), and E. serpentis in C. horridus (Fantham and Porter, 1954). Fantham and Porter also found Haemogregarina sp. and the coccidial Isospora naiae in the same specimen of C. horridus. Hull and Camin (1960) examined blood smears of 15 prairie rattlesnakes, C. viridis, for haemogregarines and all were negative. Dobell (1908) had reported rattlesnake infections of Haemogregarina crotali in C. confluentus and another Haemosporidia in C. adamanteus. Hepatozoon serpentium was found in 61.3 percent of 101 C. atrox blood smears taken during a Texas field survey (Hilman and Strandtmann, 1960).

RESULTS

Color variations of these snakes ranged from gray-pink and light brown to a dark brown phase (Fig. 2). The only two dark color phase specimens collected were north of the river and one of these snakes (Fig. 3) was the smallest and darkest snake to be observed in the study. It was collected at the 7,900 foot elevation, the highest point from which a snake of this subspecies had been reported. The light phase snakes (Fig. 4) were more common at lower elevations while snakes of darker pigmentation were more common at higher elevations.

The color on the top of the head was brown, tan, or pinktan. When present the postocular stripe was a pink-tan, and the parallel supraocular bars were tan to gray in color. The ventral color of the head was cream-white, changing to cinnamon-pink or yellow-orange on the neck. Ventral body scalation color was onion and pink-white to orange-brown. One specimen had a tan tail with dark brown spots.

The iris of the eyes were brown-black in all specimens. Body blotches were sub-rectangular to eliptical in shape and tan, brown, or black in color. In all cases the blotches had a dark brown or black border, and a background color of pink to light tan. The smallest snake, 241.3 mm in length (Table 1), had as much blotch color and over-all body pigment as that found on a large specimen which was 952.5 mm in length.

Sizes of the lateral secondary blotches varied between snakes and ranged up to and became even with the outer fringes of the dorsal body blotches.

The transverse tail rings had the same uniform coloring as the other body marks, except for the two terminal rings which were dark brown to black. Body mark and tail ring coloration of the dark phase specimens examined were the same color as the last

EXPLANATION OF PLATE I

These figures illustrate color variations and clarity of body markings on juvenile and adult \underline{C} . \underline{v} . abyssus.

Fig. 2. An intermediate brown phase adult.

Fig. 3. A dark phase juvenile.

Fig. 4. A gray-pink adult specimen. The majority of the snakes are of this color.



Figure 2



Figure 3



Figure 4

Table 1. Summary of descriptive data for C. v. abyssus collected in this study and compared to that previously reported. New data represent 29 specimens.

Characteristics	Thi	sstud	у		Published data1					
	Average	R	an	ge	Averag			lange		
Body blotches:										
number	41.2	36	-	48	42.0	36	-	48		
scales wide	9.5	8	-	11	10.0	8	-	12		
scales long scales between	3.0	2	-	4	3.0	2	-	4		
blotches	2.0	1	-	3	3.5	2	-	5		
Number of tail rings: male female	6.8 8.3			10 13	8.2			12 8		
Body length (mm): male female unidentified sex	834.0 790.6 755.6			1,168.4 876.3		684 ²	2/ ¹ -1	,000 ,025 ³ /		
Tail length (percent of body length): male female	8.1 8.8				7.3 5.9					

^{1/} Klauber, 1956 (unless otherwise indicated).

Durham, 1950.

two dark tail rings on all the other specimens. The basal segment of the rattle in each case was the dark brown or black of the last tail ring. The rattle color shaded to cinnamon-tan. Rattle numbers for 30 snakes ranged from zero to ten. Those snakes with a button had three to five rattles.

Two of 29 specimens had two rows of unkeeled scales on each side of the ventral scales. Ventral caudal scales for males numbered 22 - 28, with an average of 25. Anomalies in scale row numbers were present on three specimens. Two snakes had counts of

Wright and Wright, 1957.

Durham, 1950.

25 - 23 - 25 - 19, the 23 counts being derived equidistant between the neck and main body portions. One transverse caudal scale count numbered 21.

Seven females of this subspecies were examined and three were gravid. One specimen contained seven embryos in the left and five in the right oviduct (Fig. 5). The oviducts lengths increased one or two ventral scale lengths (9 to 18 mm) over the nongravid average of scale occupancy. Nongravid left oviducts extended between scales 139 - 148 and the right oviducts from 125 - 140. No gross histological differences were apparent between oviducts of gravid and nongravid snakes. The embryos, enclosed in a protein fiber case which resembled thin leather, had not developed to the point where sex determination could be made. Two of the snakes contained four embryos each, while a third held 12. This would give an average of seven young per litter for the three snakes if all of the young survived birth.

No tests of venom toxicity were conducted but the snakes appeared to have a low toxicity level. One specimen in captivity for 29 days was permitted to strike a mouse of the genus Peromyscus. The mouse lapsed into a semicomatose state for 8 hours before succumbing to the venom's haemolysing effect.

A large female snake was placed in a cage with an adult rock squirrel, <u>Citellus variegatus</u>. The squirrel was bitten once in the left masseter, once low in the latissimus dorsi, and twice in the left biceps femoris. After being confined with the snake for 2 days the squirrel was released to prevent its killing the snake. The squirrel showed no apparent ill effect from the bites, meaning that the venom from the snake was either injected in amounts too small to be toxic or that the toxicity level was low.

EXPLANATION OF PLATE II

Fig. 5. Left and right oviducts showing embryo placement. Fibrous covering of embryos is pronounced in the left oviduct.



Figure 5

One minute after intramuscular injection of 0.005 mg of venom in the right gracilis of a 35.5 g Swiss albino mouse, Musmusculus, breathing became heavy and continued that way until death. At 2 minutes 25 seconds the back legs showed paralysis and at 3 minutes 10 seconds there was a series of nervous tremors. Movement practically ceased until 8 minutes 20 seconds when the mouse began to run a little. The back right leg was stiff but the overall physical condition seemed improved. After 8 hours the mouse was dead. Necropsy showed massive hemorrhaging of the right biceps femoris, gracilis, semitendinosus, and left external oblique muscles. The spleen and liver had a normal appearance, the lungs showed random petechiae, all cerebrial arteries were distended, and the pelvis of each kidney was filled with clotted blood.

Four captive snakes were milked 41 times at intervals of 2 to 57 days. On 11 milkings (26.8 percent), these snakes proved to be dry or void of venom. A juvenile specimen milked three times was dry only once. The longest dry period observed was 49 days in an adult. One adult yielded 14 mg of liquid venom.

Striking and killing by the snakes occasionally occurred during the day, but ingestion took place only in the evening or at night.

The pH of four venom samples ranged from 6.8 to 7.8.

The stomach contents of 15 free living snakes were examined and 13 specimens of \underline{P} . $\underline{\text{maniculatus}}$ found. Also found were two unidentified rodents and three unidentified insects. The four captive rattlesnakes consumed 11 \underline{P} . $\underline{\text{maniculatus}}$, 19 $\underline{\text{M}}$. $\underline{\text{musculus}}$, and 4 $\underline{\text{Rattus norvegicus}}$ over a 9 month period. A lizard, $\underline{\text{Sceloporus}}$ sp., was ignored in preference to a specimen of $\underline{\text{M}}$. $\underline{\text{musculus}}$ for food. The intestines of four snakes contained pinyon needles which were apparently ingested as contaminants of

the rodent food source.

In one specimen's digestive tract there was a normal adult rattlesnake fang and a smaller fang, 9 and 2 mm in length, respectively. The smaller fang had the poison gland, muscles, and conducting tubule attached, indicating that it had been attached to a smaller snake when ingested.

After any of the captive snakes struck an animal in preparation for taking a meal they defecated three to seven fur balls and/or calcium pellets. Actual ingestion was not necessary for this reaction but the feeding intent had to be present. If a snake struck in anger or in defense no defecation followed. Fifty-seven pellets were examined for content: 23 were compacted hair, 6 contained bone fragments and hair, 22 were a combination of hair and calcium, and 6 were totally calcium salts. The feces pH ranged from 6.0 to 8.0. Bones in the feces were phalanges, femurs, mandible fragments and teeth. All bones except the phalanges were crushed, and the teeth were in most instances separated from the mandible or maxilla.

A parasitic dipteran larva, <u>Cuterebra</u> sp., was found in one snake's coelom, attached by its labial sclerites to the outer epithelium of the small intestine. The larva had no connection with the peritoneal lining and the posterior spiracular plates of the larva did not have access to air. The snake infested by the <u>Cuterebra</u> larva had marked hypertrophy of the liver and numerous necrotic foci. This snake also had 34 cysts 0.58 to 1.38 mm in diameter embedded in the mesentary near the proximal end of the duodenum. The cysts contained helminth larvae which had caused a granulomatus reaction and formed some central caseation. The cyst condition was also present in three other snakes.

Cestode infections were found in four snakes. One specimen had an infection of 47 tapeworms in the duodenum. These worms, Oochoristica sp., were 7 to 9 mm in length with irregularly alternating genital pores. Two snakes contained tetrathyridia of Mesocestoides sp. in the first 8 cm of the small intestine. One snake contained two while the other had one. The tetrathyridia were well developed and showed definite differentiation of the scolex musculature. Their total lengths ranged from 2.80 to 4.80 mm; neck width from 0.49 to 0.78 mm; neck length from 0.84 to 1.17 mm; sucker diameter from 0.12 to 0.216 mm. The fourth tapeworm infection was indicated by an onchosphere 36 μ in diameter found in a fecal flotation.

An adult nematode of the family Diaphanocephalidae was found in the fundus of one snake's stomach. The genus was probably Kalicephalus.

In fecal examinations of four live specimens, eight bipolar plugged eggs 26 μ in length were found. These eggs were either Trichuris sp., or Capillaria sp. Three embryonated ascarid eggs were also found. One was 40.8 by 28.8 μ and another was 30.9 μ in length. A mite egg 210 μ in length was also present.

Thirty live and dead snakes were examined for the protozoan parasite Entamoeba serpentis. None exhibited the intestinal ulcers and lesions characteristic of that infection and no live snakes had dysentery or feces that contained the protozoan's cysts. No coccidial oocysts were found.

DISCUSSION AND CONCLUSIONS

The colors of these snakes varied with the elevation and location rather than age as suggested by Schmidt and Davis (1941) and Wright and Wright (1957), and the body blotches and background coloring did not fade together at the same rate causing the blotches to disappear. Specimens from the eastern part of the Canyon were less distinctly marked than those found in the central part, and the dark phase specimens were found at higher elevations where the temperature was lower. This intense pigmentation could have been an adaptation to help absorb more of the sun's heat, for as Rahn (1942) pointed out, temperature may cause the skin melanophores to expand or contract and thus change the skin's color.

Slight color variations between snakes were common, but the majority were a gray-pink with well defined eliptical body blotches. Most blotches were a shade of brown and the tail rings were dark brown to black. The ventral scales were generally onion skin pink.

Rattles numbered up to ten and four snakes had retained their button rattle. All rattles were a cinnamon-tan color with the basal segment being dark brown or black. Using the supposition that three rattles were grown each year (Klauber, 1956), the amount of growth for the four snakes with buttons would be 241.3 to 463.5 mm per year. Until the time of this study the largest <u>C. v. abyssus</u> to have been measured and reported was 1,025 mm in length (Durham, 1950), and the smallest was 250 mm (Klauber, 1956). This study included specimens ranging from 1,168.4 mm to 241.3 mm.

Differences in body markings and length measurements between this study and those previously published could be attributed in part to a larger number of snakes included in this study (Table 1). Substantial differences were found in the ranges of the male and female tail ring numbers. With each tail ring and intermediate space being 10 to 15 mm in length, the female range of 3 to 13 tail rings could amount to tail length differences of 3 to 19.5 cm between snakes. The male tail length differences were 4 to 15 cm. Slight differences were noted between body blotch sizes and numbers when compared to previously published data.

Scale counts were relatively consistent with those taken at San Diego Zoo, with only minor variations being found. Two specimens had two rows of unkeeled scales on either side of the ventrals in contrast to Wright and Wright's (1957) and Klauber's (1956) one unkeeled scale row. Male caudal scales averaged 25, compared with Klauber's male snake average of 24. Those snakes with the unusual scale counts should be considered members of this subspecies because their scale anomolies were not consistent enough to cause reclassification. All of their other scale counts corresponded with the type subspecies counts.

Three female specimens were found to be gravid, their embryos ranging in number from 4 to 12. The oviduct lengths of the gravid females increased one to two ventral scale lengths over nongravid specimens and this oviduct length increase coincided with those recorded by Garrigues (1962) for \underline{c} . \underline{v} . \underline{v} iridis.

Ditmars (1912) stated that rattlesnakes stabbed the prey with their fangs, released it, and awaited its death by poisoning, but his findings do not seem applicable to these snakes in captivity. It was found after 38 attempts to milk four captive snakes that 23.7 percent of the time they were dry. While milking these captive specimens at interval lapses of 2 to 57 days, the largest amount of venom released did not exceed 14 mg

in liquid form. The average yield of dried venom for a fresh adult was reported to be 97 mg (Klauber, 1956). With the frequency of venom absence in this study, it was assumed that part of the deaths caused by these rattlesnakes might be attributed to trauma, shock, and/or hemorrhaging from the bite itself. Githens (1931) stated that rattlesnake venom markedly affected the kidneys of its prey, injuring the secretory cells which resulted in albuminuria and urine stained red by hemoglobin or blood. In this study the pelvis of each kidney was filled with clotted blood collected from the urine or from hemorrhages within the kidney.

Klauber's study of specific gravities placed C. v. abyssus venom between 1.08 and 1.09 which was the median of the crotalid venom range. Since there was a parallel relationship between specific gravity and toxicity, the venom of C. v. abyssus would be near the middle of the venom toxicity range. Extensive toxicity measurements were not made in this study but it was found that 0.005 mg of venom was lethal to a 35.5 g mouse compared to Macht's (1937) 0.1 mg for a 22 g mouse. This wide variation between lethal dosages was typical as shown by Githens' (1935) ten to one difference in a single subspecies. This toxicity difference might have been due to variations in the venom pH. Klauber's venom pH readings were acidic at 5.6 to 6.2. This study showed slightly acidic to basic pH readings of 6.8 to 7.8. Amino-cholinesterase at pH 7.8 to 8.0 and L-Amino-acid oxidase at pH 8.8 were basic for their optimum activity and these enzymes were prominent in C. viridis venom (Spector, 1956). It might be hypothesized that the higher the venom pH, the more toxic the venom.

Food specimens found in the stomachs of 15 free living snakes were 13 \underline{P} . maniculatus, two unidentified rodents, and

three unidentified insects. The rodents, \underline{M} . $\underline{\text{musculus}}$, \underline{P} . $\underline{\text{maniculatus}}$, and \underline{R} . $\underline{\text{norvegicus}}$ were all available food sources in Grand Canyon but only \underline{P} . $\underline{\text{maniculatus}}$ was present in the free living snakes. Fangs were found in the stomach contents of three of the snakes examined. Fang remains of a small rattlesnake were found in one snake suggesting either cannibalism, or that the smaller snake had been consumed while still attached to a rodent food source. Klauber (1956) had reported accidental ingestion of snakes while they were attached to small mammals, and occasional cannibalism by \underline{C} . $\underline{\text{atrox}}$, \underline{C} . \underline{r} . $\underline{\text{ruber}}$, \underline{C} . \underline{v} . $\underline{\text{helleri}}$, and \underline{C} . \underline{v} . $\underline{\text{viridis}}$.

Examination of 57 fecal pellets showed that digestion was fairly complete except for rodent hair which was found in 69.4 percent of the pellets. Bones and teeth were found in six pellets (10.3 percent). Snakes in this study did not void their feces at certain time intervals following feeding as stated by Kauffeld (1943) and Wright (1941), but did so when preparing to feed. Digestion was also not as complete as that observed by Kauffeld (1943) in C. lepidus klauberi.

Parasitism was found to occur in these snakes but not to the extent which would indicate it to be a serious limiting factor.

The dipteran larva, <u>Cuterebra</u> sp., found in one snake's coelom had not been previously reported to infest snakes.

Examination of the larva's body bristles and spiracular plates indicated that it might be a new species. There was no portal of entry, dermal warble, or other evidence that the larva infested the snake as an accidental host, but rather that the larva was in the snake's intestine. When the infested mouse was ingested the larva burrowed out of the mouse and through the snake's intestinal wall, fastening itself at that point, making the snake a paratenic host.

It is believed that the granulomatus and partially caseated cysts found in this study were formed by porocephalid larvae similar to the lingulatid cysts described by Hett (1924). Hett found lingulatid cysts in the liver, mesentery, and walls of the alimentary canal. The cysts found in \underline{c} . \underline{v} . $\underline{abyssus}$ were in the intestinal mesentery. Rattlesnakes of this study consumed $\underline{Peromyscus}$ spp. so it is possible that they could have been exposed to $\underline{Porocephalus}$ $\underline{crotali}$ infestations since $\underline{Peromyscus}$ is an intermediate host.

Cestode infections were evidenced by an onchosphere in the feces, a total of three Mesocestoides tetrathyridia in two snakes, and 47 tapeworms of the genus Oochoristica in another snake. With only one onchosphere being found in the fecal samples it would indicate that the snake was an abnormal host and was not permitting normal egg production by the worm. No feces were available from the snake harboring the 47 tapeworms. Rattlesnakes may be the intermediate host for Mesocestoides spp. but it has not been extensively substantiated in the literature.

The snake having the bot fly larva also had a hypertrophied liver with necrosis which appeared to be caused by migrating nematode larvae and/or adults. An additional cause of liver enlargement may have been congestion secondary to the nematode infection. One adult nematode was found in the fundus of a snake's stomach.

Fecal smears successfully used by Fiennes (1960) for both cestodes and nematodes gave negative results in this study. Fecal flotation examinations however showed bipolar plugged eggs which would indicate an infection of either <u>Capillaria</u> sp., or <u>Trichuris</u> sp. Three embryonated eggs similar to those of the rattlesnake ascarid, <u>Ascaridia flexuosa</u>, were also recovered in flotation examinations.

No trematode infections were found in the $\underline{C}.\underline{v}.\underline{abyssus}$ specimens examined, but $\underline{Neorenifer}$ $\underline{crotali},\underline{N}.\underline{glandularis}$, and $\underline{N}.\underline{kansensis}$ have been reported in related snakes. The absence of these flukes in $\underline{C}.\underline{v}.\underline{abyssus}$ does not mean that an infection is not possible since the intermediate hosts are tadpoles of \underline{Rana} spp. and \underline{Bufo} app., and both are available to this rattlesnake. Because of the possibility of host specificity, the ingestion of these amphibians does not assure the presence of a trematode infection however.

Thirty specimens of \underline{C} . \underline{v} . $\underline{abyssus}$ were examined and no intestinal protozoans or their infection symptoms found. This indicated that these snakes had either not been exposed or were not susceptible to infection by \underline{E} . $\underline{terrapinae}$, \underline{E} . $\underline{serpentis}$, or Isospora naise.

SUMMARY

Thirty specimens of <u>Crotalus viridis abyssus</u> were acquired and/or observed between June 6, 1962 and September 14, 1963. Despite specimen scarcity, new information on body characters, reproduction, food habits and feeding, and internal parasites was found.

Colors of these snakes ranged from gray-pink to light and dark brown. The light color phase snakes were predominant and were found at lower elevations. Body marking colors were tan, brown, or black, and the markings varied in number between snakes. Rattle numbers ranged from zero to ten. The growth rate for four snakes ranged from 241.3 to 463.5 mm per year using the theory of three rattles being grown each year. Overall scale counts were consistent with those taken at San Diego Zoo, but anomalies were found in body circumference counts and transverse caudal scale counts. Substantial differences were found between the number of tail rings, body length, and tail length percentages reported by Klauber (1956) and similar characteristics presented in this report.

Three of seven females examined were gravid with an average of seven embryos each. Oviduct lengths of the gravid snakes were 9 to 18 mm longer than those of nongravid snakes.

Venom toxicity varied among the snakes of this study. Juvenile male and juvenile and adult female \underline{c} . \underline{v} . $\underline{abyssus}$ had a lower venom potency than did adult males. Venom pH readings of 6.8 to 7.8 were above the 5.9 mean given by Klauber (1956). Four snakes milked 41 times at varying intervals of 2 to 57 days were dry or void of venom 26.8 percent of the time.

<u>Peromyscus</u> <u>maniculatus</u> was the food source most commonly found in the stomach of free living snakes. There was one indication that ingestion of another rattlesnake occurred.

Digestion was almost complete except for hair. Bones and teeth were found in 10.3 percent of the pellets examined while 89.4 percent of the total number contained rodent hair. Defecation occurred after strikes in preparation for feeding, but not after defensive strikes.

Parasitism was present but did not appear to be a limiting factor. A <u>Cuterebra</u> larva parasitized one snake as a paratenic host. Cestodes of the genera <u>Oochoristica</u> and <u>Mesocestoides</u> were present. Nematode parasites were evidenced by an intact adult of the family Diaphanocephalidae, eight bipolar plugged eggs, and three embryonated ascarid eggs. Thirty-four helminth larvae were encysted in the intestinal mesentery. No protozoan parasites were found.

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SOME ASPECTS OF BODY CHARACTERS, REPRODUCTION, FEEDING, AND PARASITISM OF THE GRAND CANYON RATTLESNAKE, Crotalus viridis abyssus.

bу

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AN ABSTRACT OF A MASTER'S REPORT

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MASTER OF SCIENCE

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ABSTRACT

The purpose of this study was to obtain data on parasites of the Grand Canyon rattlesnake, <u>Crotalus viridis</u> <u>abyssus</u>, and to elaborate on its body characters, food habits, feeding, and reproduction. Thirty of these rattlesnakes were collected in 1962 and 1963, and examined by standard reptile dissection and observation techniques.

Their color ranged from gray-pink to light or dark brown. The light phase snakes were at the lower canvon elevations. Tan. brown, or black body markings varied in number between snakes. Growth rates were 241.3 to 463.5 mm per year. Scale anomalies were found in a body circumference count of 23 and a transverse caudal count of 21 compared to normal counts of 25 and 19, respectively. Body blotch and length, and tail ring and length averages differed from previously reported data. Three of seven females averaged seven embryos each. Oviduct lengths in gravid snakes increased 9 to 18 mm over nongravid snakes. Adult males had the highest venom potency. Milking intervals for 41 milking attempts varied from 2 to 57 days, and the snakes were void of venom 26.8 percent of the time. Venom pH readings were 6.8 to 7.8. The deer mouse, Peromyscus maniculatus, was the most common food source. There was one indication that another rattlesnake had been ingested. Of 55 fecal pellets that were examined, 10.3 percent contained rodent bones and teeth while 89.4 percent had only rodent hair. Defecation occurred only after strikes in preparations for feeding. Parasites found were: Insecta (one Cuterebra larva); Cestoda (47 Oochoristica worms and three Mesocestoides tetrathyridia); Nematoda (one adult from the family Diaphanocephalidae, eight bipolar plugged eggs, and three ascarid eggs).